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TABLE OF CONTENTS

The prevention of black stem rust of wheat ..... A. F. El-Helaly 161

The role of plant residues in the etiology of root rot.
Vincent W. Cochrane 185

Effect of seed treatment on the germination of soybeans.
Helen S. Sherwin, C. L. Lefebvre, and R. W. Leukel 197

The relation of six groups of fungi to seedling diesases of sugar beets in Montana ..... M. M. Afanasiev 205

Kentucky Wonder bean plants as hosts for measuring southern bean mosaic virus activity ..... W. C. Price and Betty R. Holt 213

Comparison of two methods of pelleting onion seed in the control of smut ..... M. B. Linn and A. G. Newhall 218

Field studies on spread of the mild streak disease of black raspberries. ..... W. F. Jeffers and M. W. Woods 222

Resistance to bacterial wilt and black shank in flue-cured tobacco.
T. E. Smith and E. E. Clayton 227

Canna mosaic in the United States.
Philip Brierley and Floyd F. Smith 230

Infection of tomato fruits by Colletotrichum phomoides.
Joseph P. Fulton 235

Anthracnose of tomato ..... J. B. Kendrick, Jr., and J. C. Walker 247

Phytopathological Note 261

Botryosphaeria ribis, perfect stage of the Macrophoma causing ripe rot of Muscadine grapes ..... E. S. Luttrell

Book Review 264

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THE PREVENTION OF BLACK STEM RUST OF WHEAT

A. F. EL-HELALY

(Accepted for publication August 15, 1947)

INTRODUCTION

Wheat is the most important winter crop in Egypt. It is grown all over the country from the Mediterranean to the borders of the Sudan, and it is used entirely for local food. In 1937 there were about 860,000 feddans[1] in "the Delta" of Lower Egypt, 330,000 feddans in Middle Egypt, and 350,000 feddans in Upper Egypt (1). The chief varieties are Hindi, which are hard wheats of increasing importance, and Baladi, which are soft wheats. Sowing takes place in October and November, and harvest occurs in April and May.

Black stem rust caused by *Puccinia graminis* Pers. is the most serious disease of wheat in Egypt. It severely attacks the Hindi varieties of wheat, while the Baladi wheats are comparatively resistant or immune. It is so destructive to Hindi wheats that during epidemics in certain localities the entire crop may be lost.

Destructive epidemics of black stem rust occur during certain seasons, while other seasons are comparatively free from the disease. It was thought at one time (6) that black stem rust appeared every other year, alternating with yellow rust caused by *Puccinia glumarum* (Schm.) Erikss. and Henn., but this was found to be not the case. In most years, however, black stem rust is a limiting factor in wheat production.

Black stem rust appears, generally, in the warmer winters between February and March and may then affect yield very severely. The attack varies greatly, in intensity, from field to field.

It is believed, because the aecial stage of the disease has not yet been found in Egypt, that infection occurs each year by urediospores either hibernating, *i.e.*, overwintering, in the soil or being blown across the Mediterranean from Europe. Jones (29) by exposing, periodically, slides smeared with vaseline or glycerine on aeroplanes flying between Cairo and Alexandria at about 1000 ft. altitude, stated that the largest numbers of stem rust spores were found on slides exposed in March, about a fortnight before the first reported attack on wheat in Egypt.

OBJECT OF INVESTIGATION

The production of rust-resistant varieties, which is the ideal method of controlling rust diseases, requires several years of continuous work to produce a variety which combines resistance to rust with such desirable characters as good quality, good yield, etc. Therefore, it would be of great economic importance if direct methods of control could be devised.

[1] The feddan is equivalent to 1.47 acres.

The principal object of the present work is to investigate the possibility of preventing black stem rust of wheat by one of the common methods of plant protection.

METHOD AND MATERIAL

Various fungicides were dusted and sprayed on the plants in the field just before or at the time the first signs of the disease appeared on the crop. Dusts were applied by means of small hand dusters. Spraying was carried out by means of "Holder" sprayers or by means of "Bean Junior" spraying pumps. The former sprayers were used in small plot experiments, the latter in large plot experiments.

Latin square and randomized block experiments surrounded by wide belts of the same crop were used, and the analysis of variance was employed in analyzing the yield data of most of the experiments and in determining the significance of the results obtained.

Weight and volume of one thousand kernels taken at random to represent each treatment were also recorded in many cases.

The net profit or loss per feddan in Egyptian pounds[2] for each treatment was recorded in most cases. This was calculated taking into consideration that the feddan was given, on the average, 1500 liters of the diluted fungicide for each application. The cost of labor and of tear and wear of the sprayer for the application, in normal peace time, is about £0.25 and the price of the ardeb[3] of wheat grain is usually about £1.50.

Hindi D wheat variety, which is susceptible to stem rust, was used in most cases. It is mentioned when other wheat varieties were used. The seed was obtained from the Propagation Section of the Ministry of Agriculture. The following commercial and home-made fungicides were used:

1. Flowers of sulphur.
2. Kolodust: a proprietary colloidal sulphur dust.
3. Wettable sulphur: a proprietary sulphur spray.
4. Kolofog: a proprietary colloidal sulphur which contains 30 per cent sulphur (6 lb. Kolofog + 8 lb. chemically hydrated lime + 100 gallons of water).
5. Sulsol: a proprietary colloidal sulphur compound which contains 40 per cent sulphur by weight.
6. Amberine: a proprietary solution of polysulphidal sulphur.
7. Lime-sulphur (home-made):
   Diluted: 1 kilo quick lime + 2 kilos sulphur + 12.5 liters water.
   Concentrated: 1 kilo quick lime + 2 kilos sulphur + 6.25 liters water.
8. Bouisol: a proprietary colloidal copper compound which contains 15 per cent copper by weight.
9. Bordeaux mixture in powder form, mixture C.
10. Bordeaux mixture (home-made): 1 per cent = 4 lb. copper sulphate + 4 lb. quick lime + 40 gal. water.

[2] The Egyptian pound is valued at $4.94.
[3] The ardeb is equivalent to 5.619 bushels (U. S.).

The work to be described in this paper was carried out during the wheat growing seasons from 1935 to 1942 in the Field Stations of the Ministry of Agriculture at El-Serw, Sakha, Gemmeza, Dokki, Giza, Śeds, Mallawi, Shandaweel, and Mataana, and in the Estate of H.H. Prince M. A. Haleem at Fariskour.

OCCURRENCE OF THE DISEASE IN EGYPT

The first record of the disease in Egypt, in 1920, was reported by Briton-Jones (6, 7). He attributed the disease to *Puccinia graminis* Pers. Fahmy (See 39) reported *Puccinia graminis* Pers. on wheat in 1923. Melchers (39) found it on wheat at Giza in 1928. Jones (29) reported *Puccinia graminis* Pers. on wheat in 1935 and stated that "it is the most serious disease of wheat in Egypt."

DISTRIBUTION OF THE DISEASE

Black stem rust of wheat is usually very severe in Lower Egypt where loss in yield may sometimes amount to 50–100 per cent. It decreases gradually southwards becoming rare in Upper Egypt.

TABLE 1.—*The percentage of black stem rust infection in wheat sown on three different dates in various localities in Egypt*

Localities	Rust infection in wheat sown on		
	Oct. 15	Nov. 1	Nov. 15
	Per cent	*Per cent*	*Per cent*
Lower Egypt			
El-Serw	60	80	80
Sakha	60	70	75
Gemmeza	35	45	50
Middle Egypt			
Dokki	20	25	25
Seds	6	10	10
Mallawi	2	3	3
Upper Egypt			
Shandaweel	Tr.	Tr.	Tr.
Mataana	Tr.	Tr.	Tr.

This is illustrated in table 1 which contains data obtained from similarly treated experiments made in different localities all over the country. These experiments were actually designed to find out the effect of date of sowing on the amount of disease. Data were taken from experimental plots sown on October 15, November 1, and November 15. These dates of sowing were chosen because they fall within the period during which normal sowing takes place in Egypt.

The disease was, for the first time, very severe in the Luxor region of Upper Egypt in 1941. Study of the conditions prevailing in this region at that time and examination of the crop there showed that the disease was severe on plants which had received much water or which were growing near irrigating canals.

EFFECT OF CULTURAL CONDITIONS ON AMOUNT OF DISEASE

Observations in the field and statements of farmers indicate that the amount of the disease is influenced by the cultural conditions prevailing during the growth of the wheat plants. Factors which arise in this connection are date of sowing, number of waterings, and manurial treatment.

Date of Sowing

It has been noticed during the earlier investigations of the disease that date of sowing plays an important part in the incidence of the disease.

TABLE 2.—*The black stem rust infection and yield of wheat sown on six different dates during 1939, 1940, and 1941 at Dokki, in Middle Egypt*

Date of sowing	Rust infection	Yield per feddan	Yield	Av. weight of 1000 kernels	Av. volume of 1000 kernels
	Per cent	*Ardeb*	*Per cent*	*Grams*	*Cc.*
1939					
Oct. 9	40	9.06	187.58	36.38	27.0
Oct. 15	50	9.02	186.75	36.75	27.4
Nov. 1	70	5.79	119.88	26.85	20.3
Nov. 15	85	3.73	77.23	18.45	14.8
Dec. 1	80	1.00	20.70	8.81	8.3
Dec. 15	65	0.34	7.04	4.10	4.7
Mean	.....	4.83	100.00	.....	.....
S.E.	.....	0.307	6.36	.....	.....
Sig. diff.	.....	0.97	20.10	.....	.....
1940					
Oct. 1	20	9.01	139.19	36.20	27.5
Oct. 15	25	8.95	138.11	36.12	27.3
Nov. 1	35	8.42	129.94	34.61	26.3
Nov. 15	40	7.65	118.05	30.31	23.3
Dec. 1	50	3.79	58.49	18.39	14.5
Dec. 15	50	1.05	16.20	11.77	9.5
Mean	.....	6.48	100.00	.....	.....
S.E.	.....	0.458	7.068	.....	.....
Sig. diff.	.....	1.35	20.83	.....	.....
1941					
Oct. 1	18	5.00	90.04	34.74	26.0
Oct. 15	37	7.87	142.03	33.10	24.9
Nov. 1	50	8.08	146.00	26.36	20.9
Nov. 15	60	7.31	132.01	22.90	18.7
Dec. 1	53	3.47	62.07	14.84	13.1
Dec. 15	35	1.46	26.04	9.61	9.3
Mean	.....	5.53	100.00	.....	.....
S.E.	.....	0.375	6.78	.....	.....
Sig. diff.	.....	1.11	20.00	.....	.....

The plants of the late sowings usually have heavier attacks and give much lower yields.

An illustration of the effect of date of sowing on amount of disease and consequently on yield was clearly seen in an experiment which was designed for this purpose in 1939–1940 at Dokki. Successive sowings were made Oct. 9 and 15, Nov. 1 and 15, Dec. 1 and 15, 1939. Plots were 3×7 meters, and each treatment was repeated six times. The data obtained are in table 2 and illustrated in figure 1.

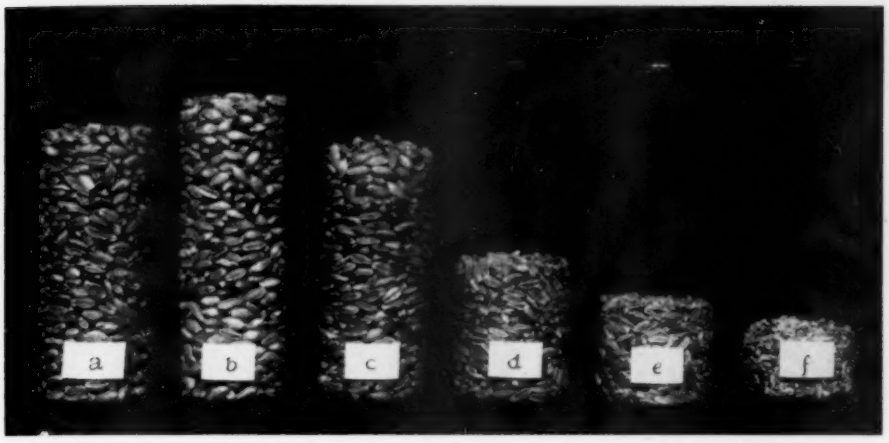

FIG. 1. Six samples, each containing 1250 wheat kernels, collected at random from crops sown on different dates in 1939—a, Oct. 9; b, Oct. 15; c, Nov. 1; d, Nov. 15; e, Dec. 1; and f, Dec. 15.

The same experiment was repeated in different localities in 1940–1941 and in 1941–1942. Table 2 also contains data obtained from the two experiments at Dokki in 1940–1941 and 1941–1942, respectively.

The weight of the yield tends to diminish with successive sowings. A careful study of the data presented in table 2 will show that, though the effect of date of sowing on yield is pronounced, the differences in yield are not always well correlated with the amount of disease. It seems that the plants of the early sowings escaped most of the ill effects of the disease. The disease appeared on the plants of the early sowings after the formation of the seed, *i.e.*, when the crop was about to ripen. This left a short time for the disease to parasitize the host and, thus, though rust was severe, it had little effect on the yield.

To find out this, the plants of the experiment of 1941–1942 at Dokki were observed closely from the time of sowing to the time of harvest. Records of the dates of the first appearance of the disease on the plants and the first appearance of the ears are shown in table 3.

TABLE 3.—*The time elapsing between heading and the appearance of black stem rust infection in wheat sown on different dates in 1941 at Dokki, in Middle Egypt*

Date of sowing, 1941	Date of the appearance of		Period between appearance of ears and rust
	Ears	Rust infection	
			Days
Oct. 1	Dec. 20	Feb. 17	60
Oct. 15	Jan. 6	Feb. 19	45
Nov. 1	Feb. 4	Feb. 23	20
Nov. 15	Feb. 17	Feb. 26	10
Dec. 1	Mar. 3	Mar. 3	0
Dec. 15	Mar. 13	Mar. 13	0

It appears from the records given in table 3 that the period between the appearance of the ears and the initial attack of the disease on the same crop diminishes gradually with the successive sowings. It was 60 days in the first sowing, 45 in the second, 20 in the third, and 10 in the fourth. The disease and the ears appeared at the same time on the plants of the last two sowings.

The ears, or the kernels, of the plants of the first sowings had, therefore, much more time to grow during the absence of the disease. The kernels were nearly mature at the time of the appearance of the disease on the crop. They almost attained, therefore, their full size, so that a normal yield was produced.

In the last sowings, the disease had, from the beginning, a very big share of the food provided by the plants for the formation of the kernels. The kernels, thus, matured abnormally and became shrivelled, and there was an enormous decrease in the yield.

TABLE 4.—*The effect of irrigations on development of black stem rust and on yield of wheat at Dokki, Egypt, in two growing seasons*

Growing season and number of waterings	Rust infection	Yield per feddan	Yield	Av. weight of 1000 kernels	Av. volume of 1000 kernels
	Per cent	*Ardeb*	*Per cent*	*Grams*	*Cc.*
1939–1940					
One, at sowing time	30	4.18	70.73	25.36	19.3
Two	50	6.59	111.51	27.25	20.8
Three	55	6.67	112.86	28.01	21.0
Four	65	6.22	105.25	26.56	20.3
Five	65	5.85	98.98	26.33	20.0
Mean	.....	5.91	100.00	.....	.....
S.E.	.....	0.586	9.92	.....	.....
Sig. diff.	.....	1.91	32.33	.....	.....
1940–1941					
One, at sowing time	30	4.71	77.09	30.12	23.2
Two	60	5.64	92.32	31.41	24.0
Three	70	6.61	108.18	33.64	26.0
Four	70	6.65	108.84	34.57	26.5
Five	70	6.94	113.58	34.75	26.5
Mean	.....	6.11	100.00	.....	.....
S.E.	.....	0.408	6.678	.....	.....
Sig. diff.	.....	1.26	8.41	.....	.....

Number of Waterings

It has been mentioned that the outbreak of the disease in the Luxor region in 1941 was much more severe in the crops which had received more water during their growth. It was also observed by Pantanelli (43) that infection was very severe in cases where much water was absorbed by the plant, in proportion to the weight of its absorptive system, during the period immediately preceding inoculation, and that an increased concentration of the nutrient solution diminished susceptibility in so far as it reduced the absorptive activity of the roots.

To find out whether the number of waterings given to the plants during

their growing season affects in any way the development of stem rust disease, two similar experiments were designed at Dokki, one in 1939–1940 and the other in 1940–1941, on 3×7 meter plots. Each treatment was repeated five times. The results are in table 4.

The amount of the disease increased with the increase in the number of waterings given to the crop during its growing period. There was no appreciable difference in the yields for the different treatments. The expected increase in yield of the crop receiving one watering, where rust was light, was not attained. This was due to shortage in the soil moisture resulting from the single watering received by the plants. The loss in yield due to the insufficient single watering was equal to, if not more than, that caused by rust in other treatments. In the first case yield was affected by lack of water, while in the other cases the plants were weakened by rust. In both cases the grain shriveled as a result of too rapid ripening of the crop.

The number of waterings seemed to have also an indirect effect on the spread of the disease. The irrigated soil usually produced a humid atmosphere around the plants themselves. This promoted germination of the spores and thus facilitated secondary infection.

Manurial Treatment

It is a general experience of farmers that nitrogenous manures increase the incidence of the disease. The value of manuring as a preventive of rust was demonstrated by many workers on plant diseases. Hicke (26) reported that wheat plants receiving potassium salt together with ammonium sulphate remained almost entirely free from infection, while those receiving only ammonium sulphate were heavily infected. Phosphoric acid and lime also tended to counteract the development of yellow rust, which had been observed to occur with particular severity on wheat following clover and other Leguminosae. Günther (23) stated that infection with yellow rust was most severe in fields where wheat followed clover and root crops, because of the excess of nitrogen and the depletion of potash and phosphoric acid in the soil. Fertilization with ammonium compounds reduced the incidence of infection but induced lodging. The greatest resistance was in crops growing on soil to which potash and phosphoric acid had been applied. Mass (36) reported that potash increased resistance to yellow rust. Finger (15) stated that although late applications of nitrogen were usually considered to increase susceptibility, yet plentiful and uniform applications greatly reduced the incidence of infection but also resulted in lodging and delayed maturity. Liberal applications of potash strengthened the plants and increased their resistance to yellow rust. Remy and von Meer (45) concluded that potassium chloride gave better control of yellow rust than potassium sulphate. There appears to be a correlation between the high chloride content of the salt (acting, perhaps, by acidification of the cell sap) and the resistance to yellow rust. Schilcher (47) found that potash and phosphorus fertilizers reduce the incidence of rust which was promoted, on the other hand, by nitrogenous fertilizers.

In order to find out the effect of manurial treatments on stem rust of wheat, an experiment was carried out at Dokki during 1939–1940, using 3×7 meter plots which had been differently manured. The treatments, for

TABLE 5.—*The black stem rust infection and yield of wheat given various manurial treatments at Dokki, Egypt, in 1939–1940*

Treatment and time applied	Rust infection	Yield per feddan	Yield	Av. weight of 1000 kernels	Av. volume of 1000 kernels
	Per cent	*Ardeb*	*Per cent*	*Grams*	*Cc.*
Calcium nitrate					
After sowing	50	4.85	90.99	25.52	19.5
Before first watering	50	6.23	116.89	29.21	22.5
One-half after sowing, one-half before first watering	60	6.94	130.21	30.18	22.8
One-half after sowing, one-half before second watering	60	5.99	112.38	28.39	21.3
One-half before first watering, one-half before second watering	60	5.71	107.13	28.16	21.1
One-third after sowing, one-third before first watering, and one-third before second watering	62	6.03	113.13	28.43	21.3
Potassium sulphate					
After sowing	50	4.85	90.99	25.52	19.5
Before first watering	60	4.80	90.06	25.45	19.4
One-half after sowing, one-half before first watering	65	5.44	102.06	27.10	20.5
One-half after sowing, one-half before second watering	55	4.81	90.24	25.45	19.5
One-half before first watering, one-half before second watering	55	4.55	85.37	25.31	19.4
One-third after sowing, one-third before first watering, and one-third before second watering	60	5.07	95.12	26.50	20.5
Superphosphate					
Before sowing	50	4.63	86.87	25.51	19.8
Farmyard manure					
Before sowing	50	5.34	100.19	26.91	20.8
Control	50	4.80	90.06	25.45	19.3
Mean	....	5.33	100.00	....	....
S.E.	....	0.465	8.72	....	....
Sig. diff.	....	1.35	25.32	....	....

triplicate plots, were as follows: Calcium nitrate, 200 kilos per feddan; Potassium sulphate, 200 kilos per feddan; Superphosphate, 200 kilos per feddan; Farmyard (Baladi) manure, 15 cubic meters per feddan; Control, nothing added.

The data (Table 5) show that there is a particular advantage in using the nitrogenous manure. The plants manured with calcium nitrate, except those of the first treatment, gave slightly better yield. There was not much difference in the amount of the disease in all treatments.

Two experiments using only the nitrogenous manure (Calcium nitrate) were designed at Dokki during 1940–1941 and 1941–1942 in order to ascertain the value of the nitrogenous manure on the incidence of black stem rust. The data obtained are in tables 6 and 7. The expected increase in yield in the manured plots was not attained because the amount of the disease on the manured plants was more than the amount on the controls.

TABLE 6.—*The black stem rust infection and yield of wheat given calcium nitrate at various times and grown at Dokki, Egypt, in 1940–1941*

Time at which calcium nitrate (200 k. per feddan) was applied	Rust infection	Yield per feddan	Yield	Av. weight of 1000 kernels	Av. volume of 1000 kernels
	Per cent	*Ardeb*	*Per cent*	*Grams*	*Cc.*
After sowing	68	2.79	105.28	13.59	11.9
Before first watering	65	2.39	90.18	12.90	11.2
Before second watering	65	2.88	108.67	13.70	11.9
One-half after sowing and one-half before first watering	70	2.76	104.15	13.51	11.8
One-half after sowing and one-half before second watering	70	2.46	92.83	13.12	11.4
One-half before first watering and one-half before second watering	75	2.45	92.45	12.99	11.4
One-third after sowing, one-third before first watering, and one-third before second watering	75	2.68	101.15	13.32	11.5
Control	40	2.75	103.73	13.39	11.6
Mean	.....	2.65	100.00	.....	.....
S.E.	.....	0.268	10.113	.....	.....
Sig. diff.	.....	0.79	29.81	.....	.....

VARIETAL RESISTANCE

The Hindi varieties of wheat are susceptible to stem rust disease, while the Baladi varieties are comparatively resistant or even immune.

In an experiment at Dokki during 1941–1942 a number of commercial varieties were tested with reference to this point. Observations also were taken from variety experiments made in the same year all over the country. The data obtained are in table 8.

Among the varieties tested under the different conditions of these experiments the least susceptible to black stem rust disease were the Baladi varieties. The Hindi varieties were much more susceptible to the disease.

USE OF FUNGICIDES FOR DISEASE CONTROL

Much work has been done in an attempt to prevent attacks of rust diseases by chemical means. Kellerman (30) was among the earlier workers

on this subject. He treated wheat, oats, and barley with flowers of sulphur, potassium sulphide, iron chloride, and Bordeaux mixture, and got negative results. Many other investigators, including Pammel (42), Hitchcock and Carleton (27, 28), Bolley (5), Cobb (9), Galloway (16), Pearson (44), and Noiret (41), followed his footsteps using different fungicides to find out whether or not the development of rust could be prevented, but they did not get definite results.

TABLE 7.—*The black stem rust infection and yield in wheat supplied with varying amounts of calcium nitrate and grown at Dokki, Egypt, in 1941–1942*

Calcium nitrate added to the feddan at the rate of	Rust infection	Yield per feddan	Yield	Av. weight of 1000 kernels	Av. volume of 1000 kernels
	Per cent	*Ardeb*	*Per cent*	*Grams*	*Cc.*
100 kilos: before first watering	21	10.88	111.00	35.37	27.4
100 kilos: one-half before first watering and one-half before second watering	21	11.14	113.06	34.57	26.8
200 kilos: before first watering	24	9.05	92.03	33.54	26.3
200 kilos: one-half before first watering and one-half before second watering	24	9.18	93.67	33.97	26.5
300 kilos: before first watering	24	9.96	101.06	32.34	26.2
300 kilos: one-half before first watering and one-half before second watering	26	9.63	98.02	33.33	25.6
300 kilos: one-third before first watering, one-third before second watering, and one-third before third watering	26	9.06	92.04	32.78	25.5
400 kilos: one-fourth before first watering, one-fourth before second watering, one-fourth before third watering, and one-fourth before fourth watering	31	9.24	94.02	32.10	25.1
Control	18	10.10	103.00	36.13	27.9
Mean	.....	9.80	100.00	.....	.....
S.E.	.....	0.798	8.14	.....	.....
Sig. diff.	.....	2.28	23.02	.....	.....

Eriksson and Henning (14) gave a detailed summary of the earlier investigations on the possibility of controlling rusts by the use of chemicals, and they concluded that, although partial control of rust is possible by the use of fungicides, practical control is not feasible.

The results of the experiments of Kightlinger (31) demonstrated clearly the possibility of controlling cereal rusts by dusting growing plants with sulphur. He pointed out that spraying is impracticable. Lambert and

Stakman (34), and Gassner and Straib (17) also recognized the impracticability of spraying for the control of rust diseases of small grains.

Bailey and Greaney (2) succeeded in controlling leaf and stem rust of wheat by dusting the growing crop with sulphur. The works of Kightlinger and Whetzel (32) and of Lambert and Stakman (33) confirmed the possibility of preventing rust by dusting with sulphur. Deutelmoser (10) recommended spraying with Bordeaux mixture for the prevention of rust of peas, beans, celery, and asparagus. Bailey and Greaney (3, 4), Sibilia (48), Güssow (24), Mencacci (40), Roussakoff (46), Lambert and Stakman (34), Lehman and Poole (35), Greaney (18, 19), Mains (38), Stakman and Person (50), and Broadfoot (8) found that rusts of wheat and oats were considerably reduced by dusting the plants in the field with sulphur (Mencacci used

TABLE 8.—*The black stem rust infection in several varieties of wheat grown at five different places in Egypt in 1941–1942*

Variety	Origin of variety	Percentage rust infection at				
		Dokki	Mazghouna (Giza)	Gemmeza	Sakha	El-Serw
Baladi 116	Single plant selection	Tr.	Tr.	Tr.	Tr.	Tr.
Baladi Bouhi	Do	8	.....	.....	.....	.....
Hindi Maarad	Do	25	.....	.....	.....	.....
Hindi D	Do	35	25	60	55	80
Hindi 62 (Dahabi)	Do	20	30	65	60	70
Kazouria	Foreign variety	15	.....	.....	.....	.....
Giza 121 (Mabrouk)	Giza 7 × Baladi 42	25	8	40	35	45
Giza 129	Do × Hindi 39	.....	3	50	70	20
Giza 130	Do × Hindi 39	.....	3	10	50	10
Giza 131	Do × Hindi 39	.....	3	5	50	5
Giza 133	Do × Hindi 12	.....	2	15	25	15
Hindi Abiad (Tosson)	Single plant selection	25	.....	.....	.....	.....

cupric sulphur), which was applied by means of hand, horse-drawn, and aeroplane dusters.

Schilcher (47) found that while Bordeaux mixture proved ineffective in combating the brown rust of wheat, some benefit was derived from unoiled calcium cyanamide dust, though accompanied by burning of the leaves. Grooshevoy and Maklakova (22) stated that dusting with sulphur can be financially sound only in regions where rusts are particularly severe and in years of heavy epidemics. The work of Greaney (20 and 21), Mackie (37), and Sibilia (49) confirmed the conclusion that sulphur dust is effective in the prevention of wheat rusts. El-Helaly (12 and 13), in testing the value of different fungicides for bean rust control, found that spraying with one-half per cent Bordeaux mixture could be considered as the most efficient and profitable method for controlling rust and chocolate spot (11) of beans in Egypt. Hart and Allison (25) used borax (sodium borate), picric acid, para-toluenesulphonylamide, and ortho-toluenesulphonylamide

for stem rust control and found that the two last named gave the most promising results. The data obtained are considered to justify further experimentation with the two toluene compounds. Straib (51) concluded that it was as yet impossible, on the basis of his greenhouse trials, to forecast the commercial possibilities of spraying with picric acid, ortho- and para-toluolsulphonamide, acridin, and 0.2 per cent borax.

Preliminary Experiments.—A preliminary experiment was designed at Giza using a susceptible wheat variety, Hindi 12, which was sown as a protective belt for a checker experiment. The plants were already slightly infected at the beginning of the treatments which commenced on March 12, 1936. Sprays were applied at weekly intervals, while dusting with sulphur was performed twice a week. Each treatment was repeated twice. Treated and nontreated plots were alternately arranged. The results taken at harvest time, May 11, 1936, are given in table 9.

TABLE 9.—*The black stem rust infection in wheat grown in duplicate plots at Giza, Egypt in 1935–1936 and treated with various fungicides*

Fungicides	Percentage rust infection	
	Plot 1	Plot 2
Nontreated	81	97
0.4 per cent Sulsol + 0.4 per cent soap	28	4
Nontreated	32	76
0.4 per cent Bouisol	5	7
Nontreated	81	88
Sulphur, 15 kilos per application per feddan	63	45
Nontreated	97	77
5 per cent diluted lime-sulphur	11	5
Nontreated	93	90
1 per cent Bordeaux mixture, mixture C	8	9
Nontreated	81	90

The data show that the effect of sprays in preventing stem rust is pronounced: the incidence of the disease was reduced about 80 per cent. On the other hand, sulphur dust had only slight effect on the disease.

This work was continued during 1936–1937 and 1937–1938 to verify the results in hand. Weight of yield was taken into consideration.

The experiments which were designed for this purpose at Gemmeza and Fariskour in 1936–1937 and at Dokki in 1937–1938 gave similar good results concerning the control of the disease. The disease was slight in these two seasons and appeared, also, late on the plants, particularly in 1937–1938.

Tables 10 and 11 present the data obtained from the Gemmeza and Dokki experiments of 1936–1937 and 1937–1938, respectively. Plots 3×4 meters were used, and each treatment was repeated three times.

The effect of the disease on the crop was slight because it appeared late on the plants of the two experiments. Nevertheless, the results obtained give a hopeful indication of the possibility of preventing black stem rust with chemicals. The following observations are worth mentioning:

1. Dusting with flowers of sulphur and with Kolodust had a very slight effect on controlling stem rust.

TABLE 10.—*The black stem rust infection and yield in wheat sprayed with fungicides. Experiments at Gemmeza, Egypt in 1936–1937*

Fungicides and concentrations	Interval between applications	Treatment commenced on February 3, 1937			Treatment commenced on March 5, 1937		
		Total no. of applications	Rust infections	Av. yield per feddan	Total no. of applications	Rust infections	Av. yield per feddan
	Weeks		*Per cent*	*Ardeb*		*Per cent*	*Ardeb*
Amberine	2	6			4		
0.2 per cent[a]			55	8.2		55	7.4
0.1 per cent[a]			55	8.5		65	7.6
0.05 per cent[a]			70	8.3		70	6.3
Dilute lime sulphur	2	6			4		
5.0 per cent[a]			15	9.6		15	9.6
2.5 per cent[a]			20	8.6		20	9.0
1.25 per cent[a]			25	8.6		40	8.6
Sulsol	2	6			4		
0.3 per cent[a]			30	8.1		50	7.8
0.15 per cent[a]			45	8.9		60	7.6
0.075 per cent[a]			48	8.7		70	8.3
Bouisol	2	6			4		
0.2 per cent			65	7.1		45	8.0
0.1 per cent			60	7.1		60	7.3
0.05 per cent			55	8.6		65	7.2
Bordeaux mixture C	2	6			4		
1.0 per cent			20	9.4		50	8.8
0.5 per cent			25	8.3		60	8.6
0.25 per cent			40	8.7		60	8.3
Amberine	4	3			2		
0.2 per cent[a]			55	8.3		65	7.7
0.1 per cent[a]			70	7.5		75	7.8
0.05 per cent[a]			70	7.1		75	7.7
Dilute lime sulphur	4	3			2		
5.0 per cent[a]			25	8.9		40	8.2
2.5 per cent[a]			40	8.7		50	7.9
1.25 per cent[a]			45	8.7		55	7.7
Sulsol	4	3			2		
0.3 per cent[a]			45	7.2		65	6.5
0.15 per cent[a]			40	8.1		65	7.1
0.075 per cent[a]			50	7.8		70	8.1
Bouisol	4	3			2		
0.2 per cent			50	8.3		65	6.8
0.1 per cent			60	8.3		70	7.0
0.05 per cent			60	7.9		70	6.7
Bordeaux mixture C	4	3			2		
1.0 per cent			40	8.8		60	8.0
0.5 per cent			45	8.2		70	7.2
0.25 per cent			60	8.7		70	7.3
Control (Not treated)	.....	.....	70	7.2	.....	70	7.2

[a] A 0.2 per cent soap was added to the fungicide.

2. A few sprays gave rather satisfactory control of the disease. Lime-sulphur seemed to be the most effective in preventing stem rust.

TABLE 11.— *The black stem rust infection and yield of wheat dusted or sprayed with sulphur compounds. Experiments at Dokki, Egypt in 1938–1939*

Treatment, sulphur compound, and amount used on a feddan	Number of applications and dates, 1939	Rust infection	Av. yield per feddan
		Per cent	*Ardeb*
Dust			
Flowers of sulphur	Three: Mar. 28,		
20 lb.	Apr. 6 and 28	40	9.57
40 lb.		40	9.49
80 lb.		35	9.81
Control		40	9.22
Kolodust	Three: Mar. 28,		
20 lb.	Apr. 6 and 28	40	9.73
40 lb.		40	9.71
80 lb.		35	9.97
Control		40	9.65
Spray			
Kolofog	One: Mar. 27		
20 lb.		40	9.25
40 lb.		35	10.19
80 lb.		35	10.15
Control		40	9.74
Kolofog	Two: Mar. 27		
20 lb.	and Apr. 5	35	10.23
40 lb.		35	10.10
80 lb.		35	10.30
Control		40	9.87
Kolofog	Three: Mar. 27,		
20 lb.	Apr. 5 and 28	45	8.74
40 lb.		35	10.20
80 lb.		30	10.74
Control		35	10.10
Wettable sulphur	One: Mar. 27		
20 lb.		35	10.92
40 lb.		40	9.48
80 lb.		40	10.03
Control		40	9.99
Wettable sulphur	Two: Mar. 27		
20 lb.	and Apr. 5	35	10.24
40 lb.		40	9.44
80 lb.		30	10.83
Control		40	9.74
Wettable sulphur	Three: Mar. 27,		
20 lb.	Apr. 5 and 28	25	11.92
40 lb.		25	11.05
80 lb.		25	11.55
Control		35	10.39
Dilute lime-sulphur	One: Mar. 27		
5 per cent		10	10.95
2.5 per cent		20	10.11
1.25 per cent		25	10.79
Control		40	9.93
Dilute lime-sulphur	Two: Mar. 27		
5 per cent	and Apr. 5	5	11.57
2.5 per cent		10	10.52
1.25 per cent		20	10.73
Control		45	9.36
Dilute lime-sulphur	Three: Mar. 27,		
5 per cent	Apr. 5 and 28	Tr.	11.96
2.5 per cent		5	11.66
1.25 per cent		15	10.91
Control		45	9.37

3. Although the difference in amount of yield between the treated and the nontreated plots was small, yet it was sufficient for us to conclude that these sprays are somewhat effective in preventing the disease. The difference was more pronounced with lime-sulphur.

This work was continued in 1939–1940 in order to study the relative fungicidal value of the available sulphur dusts and sprays and to determine which of them was the most suitable for preventing the disease. Different doses of dusts and dilutions of sprays as well as different numbers of applications were tried.

TABLE 12.—*The black stem rust infection and yield of wheat treated with flowers of sulphur or with Kolodust at weekly or fortnightly intervals. Experiments at Dokki in 1939–1940*

Sulphur compound and amount used	Total no. of applications and time between applications	Rust infection	Yield per feddan	Yield	Av. weight of 1000 kernels	Av. volume of 1000 kernels
		Per cent	*Ardeb*	*Per cent*	*Grams*	*Cc.*
Flowers of sulphur	Six: 1 week					
20 lb.		60	5.78	96.81	26.33	19.8
40 lb.		55	5.34	89.44	26.79	20.4
80 lb.		60	5.83	97.65	25.36	19.3
Control		60	5.97	100.00	26.33	20.0
Flowers of sulphur	Three: 2 weeks					
20 lb.		65	6.23	104.35	25.25	19.3
40 lb.		55	5.54	92.79	26.14	19.8
80 lb.		60	6.34	106.19	25.47	19.3
Control		55	6.38	106.86	25.73	19.6
Kolodust	Six: 1 week					
20 lb.		55	6.03	101.00	25.29	19.3
40 lb.		55	6.19	103.68	29.98	20.4
80 lb.		55	7.15	119.76	27.13	20.5
Control		55	5.35	89.61	25.51	19.4
Kolodust	Three: 2 weeks					
20 lb.		60	5.83	97.65	25.91	19.8
40 lb.		65	5.70	95.47	26.88	20.4
80 lb.		60	6.23	104.35	26.98	20.5
Control		60	5.67	94.97	25.84	19.8
Mean		.....	5.97	100.00	.....	.....
S.E.		.....	0.498	8.34	.....	.....
Sig. diff.		.....	1.438	24.09	.....	.....

Dust Fungicides.—An experiment, using 3×7 meter plots, was carried out at Dokki in 1939–1940 to ascertain the value of flowers of sulphur and Kolodust on the prevention of black stem rust disease and, eventually, on the yield. Dusts were applied at the rate of 20, 40, and 80 lb. dust per feddan at weekly and at fortnightly intervals, each treatment being repeated three times. Table 12 contains the data obtained.

The results confirmed the conclusion already mentioned. Dusting the plants with either flowers of sulphur or Kolodust had slight effect on the disease and, consequently, on the yield.

Spray Fungicides.—The results of the preliminary experiments gave

TABLE 13.—*The black stem rust infection and yield of wheat sprayed with fungicides, and the net profit or loss resulting from treatment. Experiments at Dokki in 1939–1940*

Spray material, concentration or amount used on a feddan	No. of applications	Rust infection	Yield per feddan	Yield	Av. weight of 1000 kernels	Av. volume of 1000 kernels	Net profit or loss
		Per cent	*Ardeb*	*Per cent*	*Grams*	*Cc.*	£
Dilute lime-sulphur							
5 per cent	1	75	5.54	111.23	19.10	15.0	+ 2.600
	2	15	8.47	172.50	27.27	21.0	+ 6.235
	3	5	10.55	214.86	30.88	23.3	+ 9.925
2.5 per cent	1	80	4.45	90.63	18.76	14.8	+ 0.935
	2	45	6.91	140.73	25.56	19.6	+ 4.135
	3	15	8.38	170.67	28.69	21.7	+ 5.520
1.25 per cent	1	85	4.18	85.31	16.91	13.6	+ 0.740
	2	60	6.45	131.36	21.30	16.8	+ 3.765
	3	40	6.74	137.27	24.79	19.3	+ 4.740
Control	1	95	3.40	69.24	14.28	11.9	0.000
	2	100	3.50	71.28	16.06	13.8	0.000
	3	100	2.72	55.39	13.13	10.9	0.000
Kolofog							
80 lb.	1	95	4.38	89.20	15.68	12.7	+ 0.085
	2	80	4.40	89.61	23.22	18.5	− 0.640
	3	85	4.92	100.20	18.34	15.2	− 2.490
40 lb.	1	95	4.16	84.72	13.77	11.3	− 0.175
	2	85	4.76	94.90	18.54	14.8	+ 0.300
	3	85	4.56	92.87	17.08	14.2	− 0.050
20 lb.	1	95	3.93	80.04	15.01	12.5	− 0.440
	2	90	4.76	96.94	17.43	13.8	+ 0.500
	3	85	5.16	105.09	17.79	14.3	− 0.450
Control	1	95	3.99	81.26	13.41	11.3	0.000
	2	95	3.96	80.65	13.63	11.5	0.000
	3	90	4.76	96.94	16.77	13.7	0.000
Wettable sulphur							
80 lb.	1	75	4.61	93.89	14.42	12.2	+ 0.915
	2	85	5.28	107.55	19.62	15.2	+ 0.405
	3	80	5.04	102.64	19.44	15.9	− 1.230
40 lb.	1	90	4.03	82.07	15.93	13.0	− 0.020
	2	90	5.19	105.70	17.36	14.0	+ 1.070
	3	80	5.67	115.47	19.98	15.6	+ 0.645
20 lb.	1	90	4.04	82.28	17.24	13.8	+ 0.660
	2	95	5.19	105.70	17.13	14.2	+ 1.470
	3	80	5.48	111.60	19.73	15.5	+ 0.225
Control	1	100	3.30	67.20	13.41	11.3	0.000
	2	100	3.61	73.52	13.76	11.9	0.000
	3	95	3.94	80.24	14.01	12.0	0.000
Bordeaux mixture (home-made)							
1 per cent	1	95	3.64	74.13	16.36	13.3	− 0.865
	2	80	5.57	113.44	18.71	14.9	+ 0.505
	3	70	5.87	119.55	20.55	16.5	+ 1.620
0.5 per cent	1	90	3.89	79.22	18.64	13.2	− 0.370
	2	80	4.25	86.55	18.45	14.8	− 1.235
	3	80	4.48	91.24	16.34	13.0	− 0.105
0.25 per cent	1	95	4.29	87.37	17.20	14.0	+ 0.240
	2	90	4.20	85.53	16.88	13.7	− 0.131
	3	85	4.40	89.61	13.67	11.2	− 0.040
Control	1	95	3.89	79.22	14.02	11.4	0.000
	2	90	4.58	93.27	15.47	12.7	0.000
	3	95	3.81	77.59	16.14	13.2	0.000
Mean	—	—	4.91	100.00	—	—	—
S.E.	—	—	0.610	12.42	—	—	—
Sig. diff.	—	—	1.73	35.23	—	—	—

great promise to the possibility of preventing black stem rust disease by spraying the plants in the field with fungicides. This line of investigation was continued during the wheat growing season of 1939–1940, in order to determine the cheapest, most effective and economic spray for stem rust control. Table 13 gives the result of an experiment carried out at Dokki, using 3×7 meter plots. Different numbers of sprays with different concentrations in each case were tried. The treated plots received either one

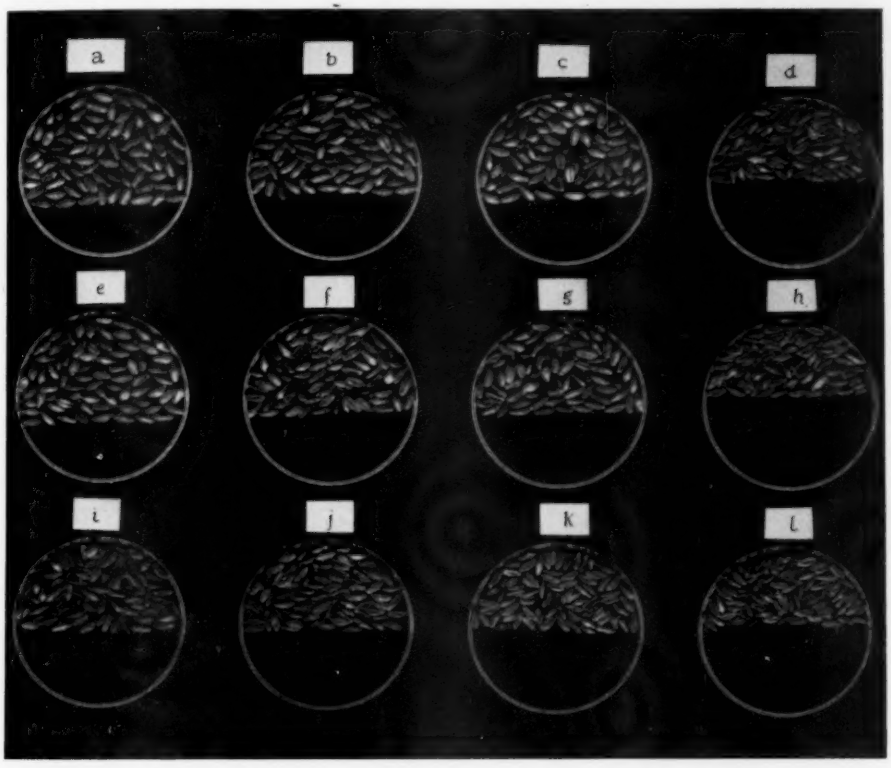

FIG. 2. Twelve samples, each containing 75 wheat kernels, collected at random from crops receiving lime-sulphur sprays in 1940. Samples a, b, and c were from crops receiving 3 sprays (March 12 and 26, and April 10) of 5 per cent, 2.5 per cent, and 1.25 per cent, respectively, of diluted lime-sulphur. Lot d was from the control plot. Samples e, f, and g were from plots receiving 2 sprays (March 12 and 26) of 5 per cent, 2.5 per cent, and 1.25 per cent, respectively, of diluted lime-sulphur. Lot h was from the control plot. Samples i, j, and k were from plots receiving a single spray (March 12) of 5 per cent, 2.5 per cent, and 1.25 per cent, respectively, of diluted lime-sulphur. Lot l was from the control plot.

spray on March 12, 1940, or two sprays on March 12 and 26, 1940, or three sprays on March 12, March 26, and April 10, 1940. Each treatment was repeated three times.

An examination of the results given in table 13 leads to the following conclusions:

1. Kolofog and Bordeaux mixture had a very slight effect on the disease

and consequently on the yield (Fig. 4, A and 3, B). There was a financial loss in most cases.

2. The return profit derived from the use of wettable sulphur fluctuated. The cost of treatment was high and, therefore, did not justify the small profit gained by the increase in yield (Fig. 4, B).
3. Excellent results were obtained with lime-sulphur. Yield increased

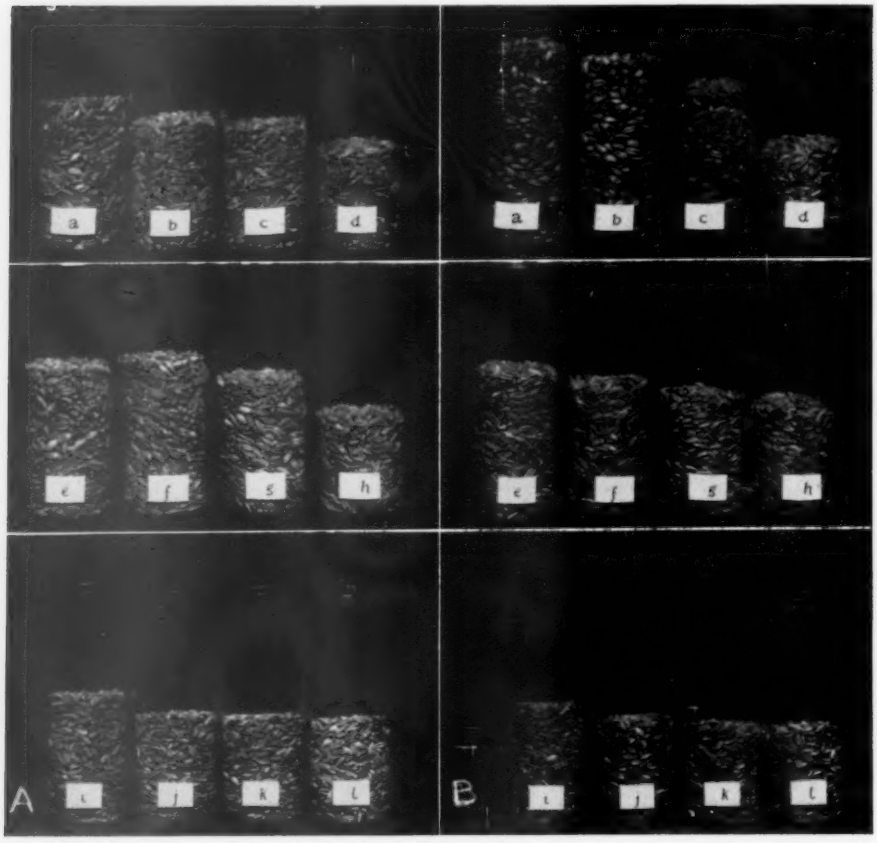

FIG. 3. Samples of 1500 wheat kernels collected at random from plots receiving dilute lime-sulphur sprays (A) or Bordeaux mixture sprays (B) to prevent stem rust.

In A, lots a, e, and i received 5 per cent lime-sulphur, lots b, f, and j received 2.5 per cent, lots c, g, and k received 1.25 per cent, and lots d, h, and l were controls.

In B, lots a, e, and i received 1 per cent Bordeaux mixture, lots b, f, and j received 0.5 per cent, lots c, g, and k received 0.25 per cent, and lots d, h, and l were controls.

In both A and B, lots a, b, and c received 3 sprays, on March 12 and 26 and on April 10, 1940. Lots e, f, and g received 2 sprays, on March 12 and 26. Lots i, j, and k received single sprays, on March 12.

to a marked degree (Fig. 2 and 3, A), and a high net profit was obtained in all cases.

4. Spraying with lime-sulphur proved to be the most satisfactory method for controlling black stem rust of wheat. It resulted in the biggest yield and the highest net profit. Yield differed according to con-

centration of spray, number of applications, and amount of disease. The net profit varied accordingly from about one to ten Egyptian pounds per feddan.

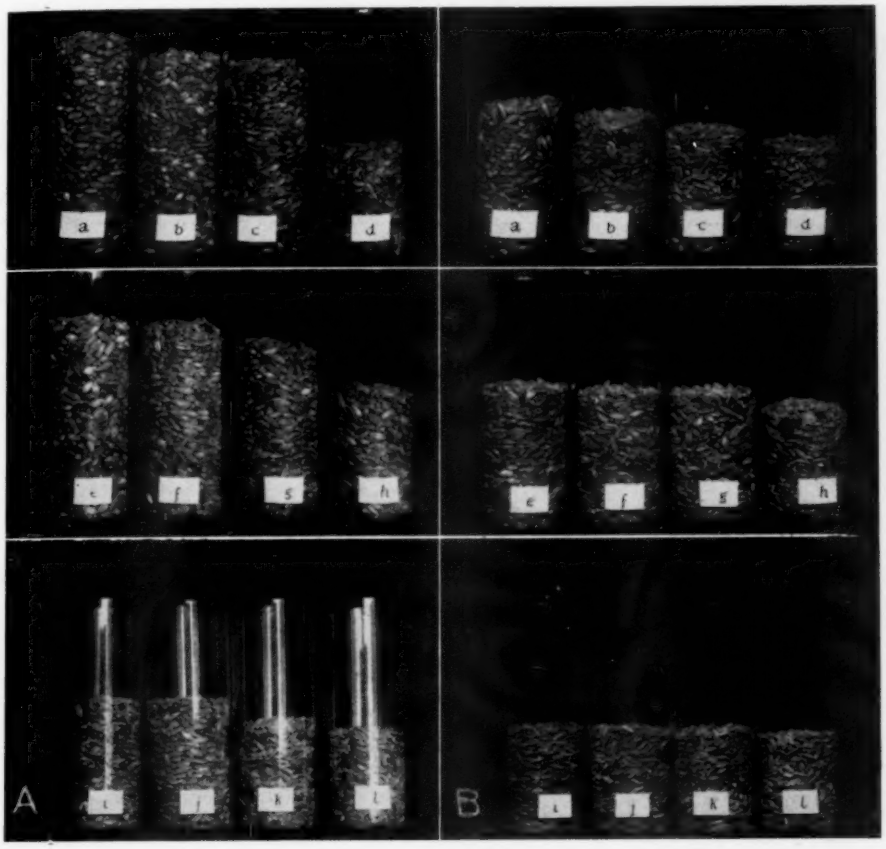

FIG. 4. Samples of 1500 wheat kernels collected at random from plots receiving Kolofog sprays (A) or wettable sulphur sprays (B) to prevent stem rust.

Lots a, e, and i received 80 lb. of the fungicide per feddan; lots b, f, and j received 40 lb.; lots c, g, and k received 20 lb.; and lots d, h, and l were controls.

Lots a, b, and c received 3 sprays, on March 12 and 26 and on April 10, 1940. Lots e, f, and g received 2 sprays, on March 12 and 26. Lots i, j, and k received single sprays, on March 12.

LIME-SULPHUR FOR THE PREVENTION OF THE DISEASE

In the experiments already described, the amount of disease was successfully reduced when plants were sprayed with 5 per cent, 2.5 per cent and 1.25 per cent diluted lime-sulphur. There was a big increase in the yield and a high net profit in all cases.

It was, therefore, advisable to study the effect of lime-sulphur on the disease with a view to determining the best concentration, the requisite number of sprays, the suitable interval between applications, and, finally, the time when the treatment should begin.

Two similar experiments, using 3 × 7 meter plots, were designed in 1940–1941, and 1941–1942 at Dokki. Each treatment was repeated six times. Tables 14 and 15 present the results obtained. The data given are highly

TABLE 14.—*The black stem rust infection and yield of wheat sprayed with concentrated lime-sulphur, and the net profit or loss resulting from such treatment. Experiments at Dokki in 1940–1941*

Strength of concentrated lime-sulphur	Date of application 1941	Rust infection	Yield per feddan	Yield	Av. weight of 1000 kernels	Av. volume of 1000 kernels	Net profit or loss
		Per cent	*Ardeb*	*Per cent*	*Grams*	*Cc.*	£.
2.5 per cent	March 5	35	9.78	115.73	31.16	23.5	+ 3.890
1.25 per cent		50	8.94	105.79	29.08	22.5	+ 2.750
0.625 per cent		55	8.62	102.01	28.91	22.4	+ 2.330
Control		80	6.78	80.23	26.77	21.0	0.000
2.5 per cent	March 25	50	8.67	102.60	29.31	22.6	+ 2.300
1.25 per cent		60	8.15	96.44	29.35	22.7	+ 1.640
0.625 per cent		60	8.12	96.09	27.45	21.2	+ 1.655
Control		80	6.73	72.54	25.55	19.8	0.000
2.5 per cent	April 12	60	8.75	98.81	28.39	21.3	+ 1.610
1.25 per cent		70	7.70	91.12	28.36	21.3	+ 0.155
0.625 per cent		75	7.31	86.50	28.29	21.3	+ 0.155
Control		75	7.27	86.03	27.22	21.0	0.000
2.5 per cent	March 5	20	10.08	118.13	32.29	25.0	+ 2.360
1.25 per cent	and 25	30	9.53	112.78	31.39	24.0	+ 1.735
0.625 per cent		45	8.93	105.68	30.55	23.5	+ 0.955
Control		75	7.72	91.36	26.94	21.0	0.000
2.5 per cent	March 5	40	8.90	105.32	29.63	23.0	+ 1.930
1.25 per cent	and	45	8.68	102.72	29.94	23.2	+ 1.910
0.625 per cent	April 12	50	8.56	101.30	29.26	22.8	+ 1.780
Control		80	6.80	80.45	25.58	19.8	0.000
2.5 per cent	March 25	40	9.95	117.51	32.12	25.1	+ 2.425
1.25 per cent	and	45	9.42	110.29	30.71	23.7	+ 1.870
0.625 per cent	April 12	50	8.81	104.26	30.43	23.5	+ 1.075
Control		75	7.52	88.99	27.11	21.2	0.000
2.5 per cent	March 5 and	5	10.26	121.42	33.13	26.0	+ 3.900
1.25 per cent	25, and	15	10.09	119.40	32.92	25.6	+ 4.005
0.625 per cent	April 12	40	8.56	101.30	29.59	22.8	+ 1.890
Control		80	6.44	76.21	26.51	20.8	0.000
Mean		....	8.45	100.00	.....	.....	.....
S.E.		....	0.494	5.846	.....	.....	.....
Sig. diff.		....	1.38	16.33	.....	.....	.....

significant and the following conclusions may be deduced:

1. Satisfactory reduction of stem rust of wheat was attained with most treatments.
2. The net profit per feddan due to the reduction of the disease varies from £1.00 to £4.00 according to the degree of the incidence of the disease, concentration of spray, number of sprays, date of initial spray, and interval between applications.
3. Spraying was successful when applied at the right time, just before or at the time of the appearance of the disease in the field (during the first half of March).

4. One spray is sometimes profitable; two and three sprays with two or three weeks interval are efficient in the reduction of the disease.
5. The most effective control and the highest net profit were attained by the use of the higher concentrations.
6. The data (Table 15) confirmed the general conclusions already put forward. This is in spite of the fact that the disease was not so severe, thus, having little effect on the untreated plants which produced, accordingly, good yield. The difference in yield per feddan between the treated and the untreated crops was, therefore, not great.
7. The net profits derived from the application of the 2.5 per cent concentrated lime-sulphur and, to a less extent, the 1.25 per cent justify their use for the prevention of the disease even in years when the disease is slight.

TABLE 15.—*The black stem rust infection and yield of wheat sprayed with concentrated lime-sulphur, and the net profit or loss resulting from such treatment. Experiments at Dokki in 1941–1942*

Strength of concentrated lime-sulphur	Date of application 1942	Rust infection	Yield per feddan	Yield	Av. weight of 1000 kernels	Av. volume of 1000 kernels	Net profit or loss
		Per cent	*Ardeb*	*Per cent*	*Grams*	*Cc.*	£.
2.5 per cent	March 9	15	12.96	96.17	35.84	26.9	−0.610
1.25 per cent		20	13.83	105.49	35.97	26.8	+0.725
0.625 per cent		25	13.31	101.52	35.27	26.5	+0.005
Control		30	13.02	99.38	34.60	26.0	0.000
2.5 per cent	March 25	10	13.89	105.94	36.23	27.1	+0.800
1.25 per cent		15	12.93	98.62	36.20	27.0	−0.490
0.625 per cent		20	12.72	97.00	36.87	27.1	−0.430
Control		30	12.95	98.07	34.26	25.9	0.000
2.5 per cent	April 12	20	13.22	100.08	34.85	26.0	−0.610
1.25 per cent		20	12.74	97.01	34.15	26.2	−0.490
0.625 per cent		25	12.18	92.09	33.95	25.6	−0.430
Control		25	13.46	102.06	34.57	25.9	0.000
2.5 per cent	March 9	2	14.15	107.09	35.04	26.3	+2.285
1.25 per cent	and 25	5	13.72	104.06	36.77	27.3	+1.870
0.625 per cent		15	12.59	96.00	35.75	27.1	+0.290
Control		25	11.82	90.16	33.33	25.0	0.000
2.5 per cent	March 9	5	13.57	103.04	35.72	27.4	+0.630
1.25 per cent	and	10	13.14	100.02	36.17	27.3	+0.220
0.625 per cent	April 12	15	12.91	98.04	34.69	26.4	−0.005
Control		25	12.34	94.01	33.86	25.6	0.000
2.5 per cent	March 25	5	13.53	103.02	35.17	25.5	−0.130
1.25 per cent	and	15	13.38	102.00	34.27	26.1	−0.125
0.625 per cent	April 12	20	13.04	99.04	34.27	26.4	−0.515
Control		30	12.81	97.07	35.92	26.7	0.000
2.5 per cent	March 9 and	Tr.	14.28	108.09	37.31	27.8	+1.170
1.25 per cent	25, and	5	13.21	100.07	37.11	27.6	−0.075
0.625 per cent	April 12	10	13.00	99.01	35.75	26.5	−0.190
Control		25	12.28	93.06	34.78	25.9	0.000
Mean		.....	13.11	100.00	.....	.....	.....
S.E.		.....	0.299	2.20	.....	.....	.....
Sig. diff.		.....	0.84	6.04	.....	.....	.....

DISCUSSION AND SUMMARY

Black stem rust of wheat is widely distributed in Lower and, to a less extent, in Middle Egypt, where it sometimes causes considerable damage and serious losses to the crop.

Certain cultural conditions influence the amount of the disease. The disease increases with the increase in number of waterings given to the plants, and, also, with the increase in the nitrogenous manure added to the soil. The plants of the early sowings usually escape the effect of the disease, because it appears on them after the formation of the seed, *i.e.*, when the crop is about to ripen.

The Hindi varieties of wheat are susceptible to black stem rust disease; while the Baladi varieties are comparatively resistant or even immune.

In testing the efficiency and the economic value of the fungicide for black stem rust prevention, it was found that dusting the growing plants in the field with flowers of sulphur and Kolodust and spraying them with Kolofog, wettable sulphur, Amberine, Sulsol, Bouisol, and Bordeaux mixture had slight effect on the disease and, consequently, on the yield. Spraying with lime-sulphur, on the other hand, was the most satisfactory method for reducing the amount of stem rust of wheat. It resulted in the biggest yield and the highest net profit. These values differ according to concentration of spray, number of applications, amount of disease and, finally, date of initial application.

The most effective control and the highest net profit were attained with the use of the higher concentrations of lime-sulphur. The increase in yield resulting from spraying with 2.5 and 1.25 per cent concentrated lime-sulphur covers the cost of tear and wear of the sprayer, the cost of material used and also labor, and finally leaves, in addition, some profit to justify its application. It is apparent that even in years when stem rust is slight (*e.g.*, 1941–1942) spraying with the two said concentrations will control rust satisfactorily and profitably. Spraying with each of the two said concentrations could therefore, be considered as the most efficient and profitable method for controlling stem rust of wheat in small areas in Egypt.

Entirely satisfactory results were always obtained by applying the initial spray to the plants when rust was first detected in the district, and then by thoroughly protecting the plants until they were practically ripe.

Although one spray gave more or less satisfactory control of the disease, yet one or two other sprays with an interval of two or three weeks were profitable. Spraying should be started just before or at the time of the appearance of the first signs of the disease in the field. Too early and too late spraying had little effect on the prevention of the disease and, therefore, was of little economic value.

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THE ROLE OF PLANT RESIDUES IN THE ETIOLOGY OF ROOT ROT[1]

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The incorporation of plant residues into the soil is known to affect, if not indeed to determine, the occurrence and severity of certain root rot diseases of plants. The types of organic matter present influence, in a manner not yet understood, the root rot of corn (17), the brown root rot of tobacco (1, 6), and the black root rot of strawberry (9).

The present investigation is based on the hypothesis that some root rots are initiated by the direct toxic action of plant residues; the hypothesis implies that the activities of soil organisms are secondary and are incident upon an initial injury which is of chemical origin.

Root rot, even so considered, remains a disease, not a symptom. Disease may be defined as an active, dynamic response by the plant to a set of abnormal environmental conditions; a nematode or microbial pathogen may or may not be a feature of this environment. A symptom is defined in static, rather than dynamic, terms; it is a visible, or at least measurable, abnormal state of the plant resulting from its prior response to the abnormal environment.

The germ theory of disease has without question led to results of the greatest theoretical and practical importance. Among the root rots there are indubitable cases of a one-to-one correspondence of disease and microbial pathogen, as in the take-all disease of wheat and the Phymatotrichum root rot of cotton. However, the root rots of strawberry and of pea are just as clearly not associated with the action of one or even a few microorganisms; the number of fungi described in the literature as causally related to these diseases is so large as to throw doubt on the whole method of approach.

Because of the common association of the word "rot" with the action of microorganisms, it might seem preferable to use a more neutral term in discussing root injury of chemical origin. It seems, however, that the same considerations apply here as in the case of the concept of disease. Most rots are caused by organisms, but exceptions occur, as in the case of autolysis after freezing. In the same way, the hypothesis of a chemical origin of certain root rots does not require a new term, only a realization that rots are not required by the very definition of the word to be caused by microorganisms.

The effect of plant residues on root rot may be, obviously, either to decrease or to increase disease incidence. With the former case we are not at present concerned, except to note that organic matter is known to decrease

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the severity of Phymatotrichum root rot (12) and the take-all disease of wheat (7). In the latter case—increase in disease—there are at least four possible theories of the mode of action: (1) direct stimulation of the growth or activity of a pathogen; (2) changes in the microbial balance of the soil or the rhizosphere; (3) depletion or unavailability of soil nutrients during microbial decomposition of the added residue; and (4) the direct toxic action of the residue or its decomposition products. It is the last-named hypothesis that the experiments reported here are designed to test.

The early work of the Bureau of Soils on "soil toxins" (18) has been justly criticized (15) and is not of itself convincing. However, several recent reports (2, 3, 16) have demonstrated that certain constituents of normal plants may be toxic to the same or other plants.

Collison (4) and Collison and Conn (5) demonstrated that extracts of wheat straw were toxic to young seedlings in water culture, causing stunting and root discoloration. The experimental design eliminated nitrogen deficiency, acidity, and oxygen depletion from consideration as causes of the symptoms. The authors concluded that specific chemicals present in the straw were responsible for plant injury; composting of the straw resulted in their disappearance.

Extracts of partially decomposed timothy have been reported by Doran (6) to cause injury to tobacco roots; Gries (8) found that extracts of decomposing red clover were toxic to strawberry roots. In both these cases the toxicity was believed to result from microbial action on the plant residue involved. Newton and Young (14) reported preliminary data indicating that extracts of undecomposed alfalfa exert inhibitory effects on the growth of wheat in sand culture; Valleau *et al.* (19) found that the addition of corn roots to sand cultures of tobacco caused a root rot; Matthews *et al.* (13) were able to induce browning of tomato roots by the incorporation into the soil of timothy roots.

As a necessary preliminary to experimental test of the hypothesis that chemicals present in plant materials may cause root injury of the type under discussion, a bio-assay was devised which, it is believed, offers some promise as a tool in further research. Such an assay, it was realized, must be free of the complications introduced by microbial activity during the experiment and must in addition be independent of changes in available nutrients. If these two conditions are met, it is possible to eliminate from consideration the first three of the theories mentioned earlier, however applicable they may be in other cases.

The present paper describes the bio-assay method and the results of its application to study of the toxicity of certain plant residues before, during, and after decomposition.

MATERIALS AND METHODS

Plant residues studied included soybean (variety Manchu), sweet corn (variety Golden Cross Bantam), ladino clover, and perennial ryegrass.

Plants were harvested (tops only) in late fall, air-dried, ground coarsely, and stored in covered stone crocks.

Each plant residue was allowed to decompose at two moisture levels. The "dry" series was decomposed at a moisture level equivalent to 50 per cent of saturation; this value ranged from 63.2 per cent for clover to 80.2 per cent for corn. In this series the residues were incubated in crocks with loose covers. Moisture contents of all samples were determined weekly and adjusted when necessary to the original level. In the "wet" series dry plant material and tap water were added to Mason jars in the ratio 1:20; in this case the solids were completely submerged.

Decomposition proceeded without the addition of soil as inoculum; preliminary experiments showed that the addition of one per cent soil had no significant effect on the number and types of microorganisms present two weeks later. The temperature of incubation was 24°–27° C., with the exception of certain experiments carried out at 56° C. Samples for determination of pH, microbial populations, and toxicity were taken before the start of decomposition, 3 days later, at weekly intervals for six weeks, and at three-week intervals until the close of the experiment.

Bacteria, actinomycetes, and spore-forming bacteria were counted on dilution plates of Bacto nutrient agar after incubation for ten days at 24°–27° C. Yeasts and fungi were counted on acidified (pH 4.0) Bacto potato-dextrose agar after four days. The numbers of thermophilic bacteria and actinomycetes were determined by counting dilution plates of nutrient agar after two days at 56° C. The population of anaerobic bacteria was measured by the extension dilution method in a reducing agar medium.

The effects of plant residues on roots were assayed by a method developed for the purpose. Radish seed (variety Early Scarlet Globe) was germinated on moist filter paper. After 72 hours seedlings with radicles 2–3 cm. in length were placed in shell vials containing the extracts to be tested. After eight hours at room temperature the seedlings were removed for grading.

Radish seed offers several advantages for this assay. Germination is rapid; the abundance of root hairs makes for quick absorption of soluble materials; and the injury is of such a nature that accurate measure of it can be made rapidly. Of the other plants tested, both spinach and pea showed the same reaction to plant residues but were less easy to manipulate; onion seedling roots, on the other hand, failed to respond to plant tissue extracts.

Extracts were prepared by soaking the plant tissue to be tested in boiled and cooled tap water for 20 minutes; the solids were then filtered off. Two concentrations were prepared in every case: one gram (dry basis) per 20 ml. of water, and one gram per 40 ml. In all experiments the control consisted of roots of the respective plants soaked eight hours in boiled tap water.

The basis of the assay was the browning of the radish root caused by certain plant tissue extracts. For each of 20 roots an estimate was made

of the fraction of the root length which had been discolored by the extract. According to the grading system devised by Horsfall and Barratt (10), this proportion was expressed as a grade; the mean grade for each lot of 20 roots was then converted to "percentage browning" by reference to a prepared calibration curve.

Only brown discoloration was considered; the color ranged from light to dark brown. The intensity of color usually decreased as the fractional root length discolored became smaller, but no effort was made to separate treatments on the basis of color intensity. All plant extracts, whether causing browning or not, induced a water-soaking of the root tissue.

RESULTS

Microbiological Population Changes

Counts of the common soil microorganisms were made at each sampling period. Results conformed in general with those of other workers (20) and need not be presented in detail. As will be brought out in the next section, toxicity of residues decreased with time, and this is believed to be associated with decomposition; but further correlation of injury with microbial populations was not evident. This is particularly striking in view of the very different floras occurring at the two different moisture levels and at the different times of sampling. In the "dry" series (50 per cent saturation), ammonification and the resultant high pH encouraged the development of bacteria and actinomycetes. In plant material submerged in water the growth of acid-forming bacteria was followed by profuse development of fungi; utilization of acid by the fungi resulted in a slow rise in pH and a gradual replacement of fungi by bacteria. The nature of the plant residue naturally affected the composition of the microflora, but again there was no apparent relation to root rot.

Apart from this negative finding, there is one point which is of interest with regard to the microbial flora of decomposing plant residues. West and Hildebrand (22) characterize the breakdown of soybean tissue as "fermentative" and that of red clover as "putrefactive," with, in the former case, a low pH and low bacterial population relative to the latter. These differences in type of breakdown were believed to have some relation to the amount of strawberry root rot in soils to which these residues were added.

The microbiological results in the present study do not bear out this contention; the pH was found to be more dependent on the moisture level than on the type of plant material. Small differences in pH did occur as a reflection of nitrogen content in cultures where ammonification was possible.

An experiment modelled after that of West and Hildebrand was designed further to explore the microbial factors involved in the decomposition of clover and soybean. Fresh soybean and red clover tissue, from plants grown 6 weeks in the greenhouse, was finely chopped and 2.5 gm. of

each (approximate 0.5 gm. dry matter) were placed in 50 ml. of tap water in a prescription bottle; soil (0.5 gm.) was added to each culture. After 14 days at 28°–30° C., pH and bacterial numbers were determined. The data, summarized in table 1, do not support the generalization that soybean tissue undergoes an "acid" or "fermentative" breakdown with low bacterial numbers. Under the conditions of this experiment soybean tissue after 14 days of decomposition was slightly more alkaline and supported a slightly larger bacterial population than red clover tissue.

Other experiments showed that the acid type of breakdown may be induced with either plant material if larger amounts of the dried residues (1.0–2.5 gm. per 50 ml.) are added to water in the same way. In all likelihood, the beneficial effect of soybean on strawberry root rot is to be ex-

TABLE 1.—*General characteristics of the decomposition of soybean and red clover tissue after 14 days at 28°–30° C.*

Plant[a]	pH		Bacteria per ml.[b] (Total No. × 10^{-6})
	Initial	Final	
Soybean	6.61	7.60	596.7
Red clover	6.68	7.25	80.7

[a] 2.5 gm. fresh tissue plus 0.5 gm. soil in 50 ml. tap water.
[b] Determined by dilution plating on Bacto nutrient agar.

plained in some other manner than the mechanism postulated by West and Hildebrand.

Toxicity of Plant Residues

Cold-water extracts of plant residues were tested for possible injurious effect on radish roots as described above. Data for several of the residues appear in figures 1 and 2.

Extracts of undecomposed ladino clover proved to be toxic, as shown by the high response at zero time (Fig. 1). Clover tissue allowed to decompose at 50 per cent of its moisture-holding capacity became slightly more toxic to roots during the early stages of decomposition; it is not certain whether fluctuations over short periods are significant, but the general trend of toxicity was upward for 5 weeks. From this time on, toxicity decreased rapidly, reaching a very low level at 9–12 weeks.

Extracts of clover decomposed at the high moisture level lost their toxicity to roots more rapidly than extracts from the drier series; the same fluctuations in toxicity early in the decomposition period were noted.

While, as pointed out previously, the pH data show that the two moisture levels governed two very different types of decomposition, in both cases the overall picture is one of destruction of the high initial toxicity, modified by microbial effects acting over short periods of time.

The other legume under test, soybean, had, in contrast to the clover, no detectable initial toxicity to radish roots. There was apparent in the early

stages of decomposition at both moisture levels a very slight toxicity presumably resulting from microbial activity. The maximum injury from a 1:20 extract did not, however, exceed 3 per cent, and the toxicity disap-

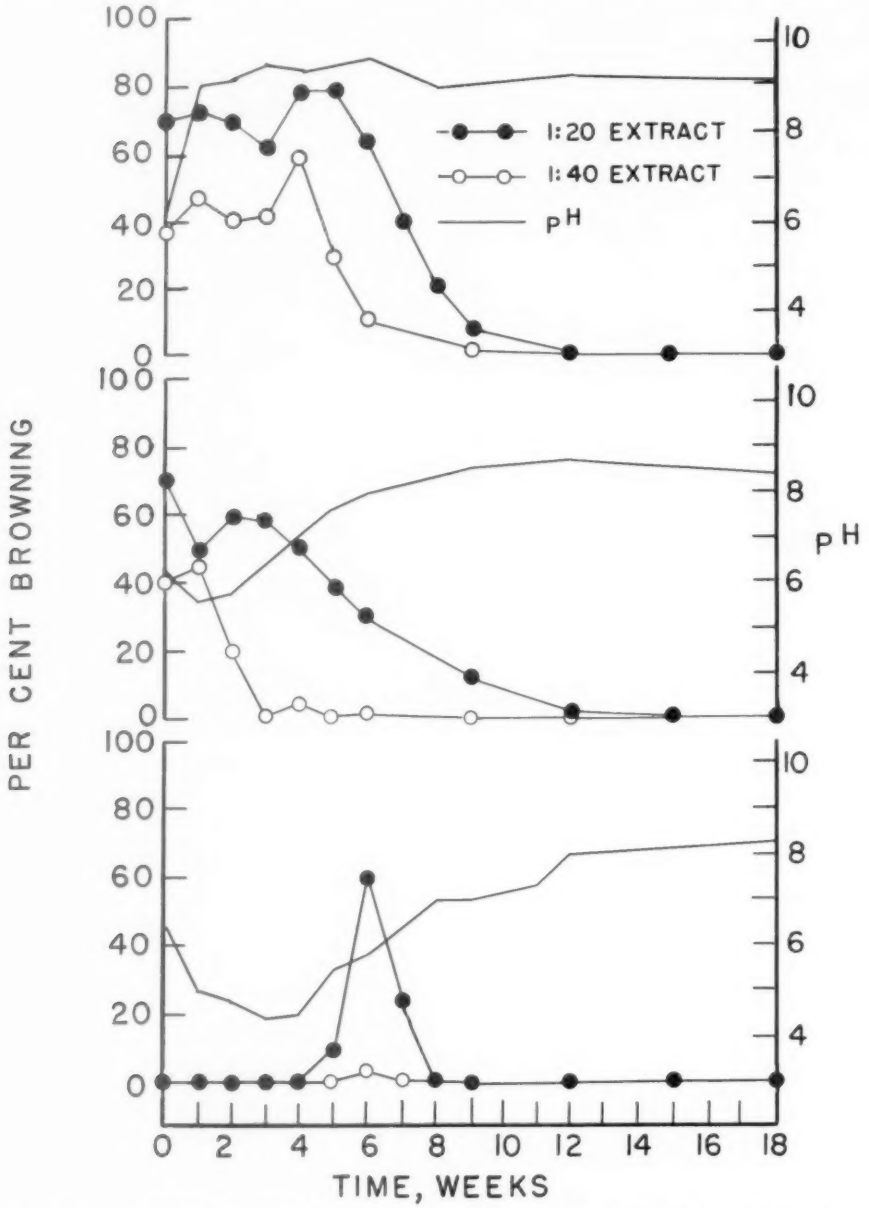

FIG. 1. Percentage browning of radish seedling roots in extracts of (1) clover tissue decomposing at 50 per cent saturation (top); (2) clover tissue decomposing under water (middle); (3) corn tissue decomposing under water (bottom).

peared in 4 weeks in one case ("dry" series), in 2 weeks in the other ("wet" series).

Extracts of undecomposed corn stover were not at all injurious to radish roots. Similarly, extracts from corn decomposed at 50 per cent of saturation were not toxic at any time over the 84-day period of observation.

In the corn stover decomposed under water, there was, as shown in figure 1, a period (28–42 days) during which extracts caused very noticeable injury to root tissue. This followed a period of minimal pH and the acidity may have been instrumental, directly or indirectly, in causing

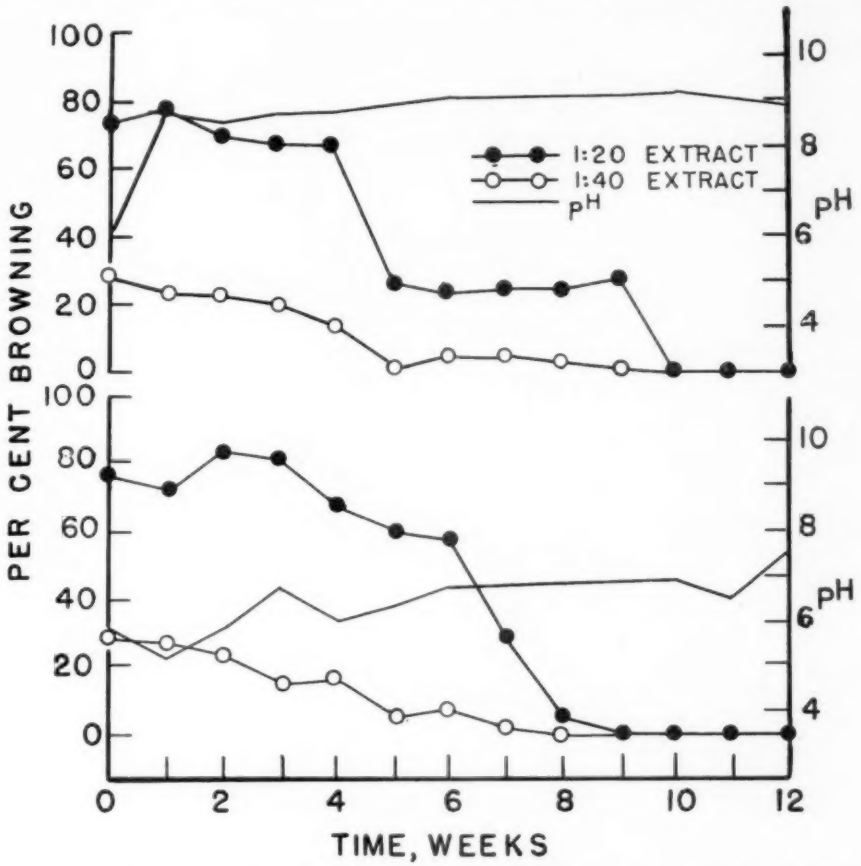

FIG. 2. Percentage browning of radish roots in extracts of ryegrass tissue decomposing at 50 per cent saturation (top), and in extracts of ryegrass tissue decomposing under water (bottom).

the injury. The effect is ascribed to microbial action, whether or not the acidity was a factor; at this stage in the decomposition fungi formed the predominant element in the microbial flora, and a steep decline in the number of fungi was coincident with the disappearance of injurious effects. It should be pointed out that the symptoms of injury to radish roots were not precisely similar to those caused by extracts of clover or ryegrass; the color of the affected tissue was more grayish, and there was a translucence not previously noted.

Extracts from undecomposed ryegrass were very injurious to radish roots, 80 per cent of the root length being damaged. As shown in figure 2, decomposition at both moisture levels resulted in progressive loss of toxicity, more rapid in the "wet" cultures than in the "dry." In the former case there was at 14 days a slight increase in injury from the 1:20 extract; since this increase did not occur with the 1:40 extract, it was probably of no significance. The course of pH change was similar to that in other plant materials and could not be correlated with changes in the toxicity of extracts.

The hypothesis that the disappearance of toxicity of clover and ryegrass residues is the result of microbial decomposition suggests that the process may be accelerated at high temperatures. Such conditions would

TABLE 2.—*The effect of decomposition at 56° C. on the toxicity of plant materials to radish roots*

Plant material[a]	Time, days	pH	Number of bacteria[b] (Total No. × 10^{-6})	Percentage browning of radish roots	
				1:20 Extract[c]	1:40 Extract[c]
Corn	0	6.52	< 0.001	0.0	0.0
	3	7.81	14.3	0.0	0.0
	7	8.00	2430.0	0.0	0.0
	14	7.68	4320.0	0.0	0.0
	21	8.39	2700.0	0.0	0.0
(Control)[d]	21	6.74	.....	0.0	0.0
Ryegrass	0	5.78	0.001	71.0	22.5
	3	7.06	48.0	49.0	13.5
	7	7.49	894.0	6.3	0.0
	14	6.97	1170.0	3.1	0.0
	21	7.45	787.0	0.0	0.0
(Control)[d]	21	5.63	.....	61.5	20.4

[a] Decomposed at a moisture content of 50 per cent of saturation.
[b] Determined by dilution plating on Bacto nutrient agar.
[c] Dry basis.
[d] Held at 56° C. for 21 days without addition of water.

approximate those of a compost pile during the period of active microbial thermogenesis. It is a commonplace of agronomic practice that composted organic matter is of greater benefit to crops than the same material before decomposition. Several factors probably contribute to the beneficial effect of composting, notably the preliminary breakdown of complex carbon compounds whose decomposition in the soil would result in a locking up of nitrogen. It seemed of interest, however, to determine the effect of compost-pile temperatures on the breakdown of the toxic factors under consideration in the present work.

The effect of decomposition at high temperature (56° C.) is shown in table 2. Samples of corn stover and of ryegrass were adjusted to a moisture level equivalent to 50 per cent of their water-holding capacity and incubated at 56° C. The moisture content was kept constant by daily deter-

minations of dry matter and addition of the required amount of water. At the high temperature dry matter losses were considerable, *e.g.*, about 40 per cent of the dry matter of ryegrass was lost in 14 days.

The toxicity of ryegrass to roots dropped very quickly as the result of high-temperature decomposition; at 21 days no injury could be noted with a 1:20 extract. At room temperature (Fig. 2), toxicity was still detectable after 84 days of decomposition.

The controls for the experiment on thermophilic decomposition were dry ground samples of the plant material incubated at 56° C. for 21 days. These controls were not, obviously, entirely satisfactory; conceivably the injurious material in ryegrass may be more sensitive to high temperature in the presence of water than in its absence. The similarity to decomposition

TABLE 3.—*The influence of the age of the plant on the toxicity of its water extract to radish seedling roots*

Plant	Date of collection	Age, days[a]	Percentage browning of radish roots[b]
Ladino clover	June 30	.....	49.5
	July 15	.....	56.0
	July 31	.....	25.0
	Aug. 15	.....	49.5
	Aug. 31	.....	73.2
Soybean	June 1	40	0.0
	June 15	55	0.0
	June 30	70	0.0
	July 15	85	0.0
	July 31	101	0.0
Corn	June 15	36	0.0
	July 15	66	0.0
	Aug. 15	97	0.0

[a] The ladino clover was planted the previous year.
[b] Radish seedlings immersed 8 hours in a 1:20 extract (dry basis) of the dried and ground tissue.

at room temperature is close, however, and it may be postulated that during the high-temperature decomposition the factor in ryegrass responsible for root injury is destroyed by microbial action.

In summary, extracts of ryegrass and of ladino clover were injurious to radish seedling roots; similar extracts of corn stover and of soybean tissue were not injurious. Decomposition resulted in a progressive decline in the toxicity of clover and ryegrass extracts; this decline was interrupted at times by secondary increases in toxicity caused apparently by microbial action, and was more rapid at 56° C. than at room temperature.

It will be noted that the two residues yielding toxic extracts were collected as green plants, the corn and soybean as dead plants. Waksman (21) found that young plants are higher in water-soluble constituents than are mature plants. The possibility exists therefore that the differences observed between plant materials are attributable to their age rather than to specific characters, since the test for toxicity depended on a water extraction.

The experiment reported in table 3 was designed to ascertain the influence of age of plant on the toxicity of water extracts to radish roots. The data show that neither young soybean nor young corn plants yield toxic water extracts. The toxicity of extracts from clover plants of different ages was variable but did not show any consistent effect of age of plant.

DISCUSSION

The laboratory assay used in the determination of toxicity of plant residue has obvious limitations. There are, however, two types of correlation with field experience which encourage belief in the validity of the assay.

First, the type of injury to radish seedling roots was very similar to that known in field root rots of other plants: watersoaking, browning, and death of tissues from the tip downward.

Second, and more important, there is a correlation with field experience in that soybean residues, known to reduce strawberry root rot (9) and corn root rot (17), were not injurious to radish. Conversely, clover residues, which generally increase root rot in soil (11, 17) proved to cause severe damage to radish root cells. The failure of corn residues to have a toxic effect may be a result of the fact that only stalks were used. Valleau *et al.* (19) found that previous corn crops increase tobacco root rot, and that dead corn roots are toxic to tobacco in sand culture.

The effect of extracts of plant residues on radish roots cannot be ascribed to nutrient deficiencies, to invasion of the roots by specific pathogens, or in all likelihood to microbial activity in the extract itself. The time period of 8 hours was too short to permit any of these factors to be operative. The injury therefore has the aspect of direct toxicity of a chemical or chemicals present in the cold-water extract of certain plant tissues.

It is important to note that the most injurious plant materials, clover and ryegrass, were so from the beginning. Microbial activity may have caused short-term increases in toxicity, but over the long run the decomposition of these materials resulted in concomitant destruction of the toxic factor. Decomposition by thermophilic organisms at 56° C. accelerated the destruction.

Undecomposed plant materials have occasionally been reported to cause injury to seedlings. Extracts of dried wheat straw and alfalfa hay were found to cause stunting of pea, corn, and barley seedlings (5); similar results have been obtained with alfalfa by other workers (14). These reports, together with the data here presented, suggest that, in studies of the effect of decomposing crop residues on plant growth, adequate controls must be included so that the effect of the plant material itself will not be interpreted as an effect of decomposition products.

A further artificial feature of the experiments here reported is the use of plant residues as such, when it is known that the concentration of such residues in soil is normally less than 10 per cent. To this objection it may

be argued that the extracts actually tested were dilute, and that in the soil local accumulations of undecomposed organic matter may occur in the vicinity of growing roots and serve to initiate root rot.

SUMMARY

Direct injury to root tissue by plant extracts can be assayed by exposing the radicles of 72-hour-old radish seedlings to the action of cold-water extracts of the ground plant tissue.

Extracts made from undecomposed ladino clover and from ryegrass caused browning of radish roots in 8 hours. Decomposition of these residues at two moisture levels resulted in a progressive disappearance of the factor causing the injury, although microbial action brought about temporary increases in toxicity. Decomposition of clover tissue at 56° C. accelerated the destruction of the toxic factor, presumably as a result of the activity of thermophilic bacteria. Apart from this case, studies of microbial populations in the decomposing residues failed to establish any significant association of particular groups of microorganisms with the appearance or disappearance of root injury.

Extracts from undecomposed corn stover did not cause root browning; microbial action under anaerobic conditions caused a transitory appearance of injurious activity.

Extracts of soybean tissue did not cause discoloration of radish roots, whether tested before, during, or after decomposition.

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EFFECT OF SEED TREATMENT ON THE GERMINATION OF SOYBEANS

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The practice of treating soybean seed with fungicides to improve emergence, stand and yield is comparatively recent, and the results obtained from treated seed planted at different locations vary considerably. In general, however, seed treatments frequently have increased stand but rarely have increased yield.

In a recent paper, Hildebrand and Koch[2] reviewed the literature on the subject so well that a review need not be repeated here. They reported that with poor quality seed, treatment with Spergon increased emergence and yield. Under the conditions of their experiments, Spergon was consistently more effective than Arasan and Fermate in reducing disease or abnormality in early-season stands.[3]

As environmental factors influence results obtained in field experiments, it seemed worthwhile to conduct seed treatment tests in constant temperature rooms where certain environmental conditions or other variables could be controlled, and to compare results from these tests with results from certain plantings made in the field.

The experiments were conducted in two series, Series I with twelve seed lots of various varieties from different places in the Southern States and Illinois, and Series II with a single seed lot, Wood's Extra Early Yellow, from Virginia. In both of these series, portions of each seed lot were left untreated or were treated separately with Arasan (tetramethyl thiuram disulphide) or Spergon (tetrachloro parabenzoquinone) at the rate of two ounces per bushel, or with New Improved Ceresan (ethyl mercury phosphate) at the rate of one-half ounce per bushel. The seed in Series I was treated March 10, 1945, and two to three weeks later portions of each lot, treated and untreated, (designated Series I–A) were planted in constant temperature rooms held at 15°, 20°, 25°, and 30° C., respectively. Other portions of five of these twelve seed lots, treated and untreated, (designated Series I–B) were stored at room temperature in loosely covered glass jars and similarly planted in 1946, one year after treatment. In addition, after 15 months' storage, five similarly treated and untreated seed lots (designated Series I–C) were planted May 24, 1946 in the field at Beltsville, Maryland, 100 seeds per row, in 8-foot rows, replicated four times.

[1] Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, Beltsville, Maryland. The authors wish to express their appreciation to Dr. A. G. Johnson for critical reading of the manuscript.

[2] Hildebrand, A. A., and L. W. Koch. Soybean diseases in Ontario and effectiveness of seed treatment. Phytopath. **37:** 111–124. 1947.

[3] Hildebrand, A. A., and L. W. Koch. Seed treatment and other tests with soybeans in Ontario. (Abstr.) Phytopath. **36:** 401. 1946.

The seed used in Series II was received in the spring of 1946. It had been grown in 1945 near Tidewater, Virginia and stored in an elevator. Seed of this lot was of poor quality and had been submitted to the writers because of the high percentage of discolored seeds.

The seed of Series II was separated into three classes according to the degree of discoloration: (1) beans with at least half of the area of the seed coat of each discolored; (2) those with less than half of the seed coat discolored; and, (3) those without discoloration. In classes (1) and (2), *Cercosporina kikuchii* Mats. and Tomo. was readily isolated from the beans with purple blotches, and oospores of *Peronospora manshurica* (Naoum.) Syd. formed conspicuous whitish crusts on many others. Portions of each of the three classes were left untreated or were treated and handled in the same manner as those in Series I, except that in Series II–A only one planting was made in the constant temperature rooms, and in Series II–B

TABLE 1.—*Influence of seed treatments at specific temperatures. The results with all seed lots were combined*

Treatment	Average number of seedlings emerged from 50 seeds in							
	Series I–A at[a]				Series I–B at[b]			
	15° C.	20° C.	25° C.	30° C.	15° C.	20° C.	25° C.	30° C.
Arasan	34.05**	37.00**	37.65**	36.87	6.87	19.87*	29.80**	28.40**
Spergon	30.54**	34.46**	36.26**	35.73	6.60	17.60	25.80**	27.47**
New Improved Ceresan	32.05**	36.94**	38.28**	36.92	13.93**	26.33**	31.60**	29.93**
No treatment	23.78	27.32	30.23	35.08	5.07	15.60	17.40	13.93

* Significant at the 5 per cent level (1.98 required for I–A, 3.39 for I–B).
** Significant at the 1 per cent level (2.61 required for I–A, 4.48 for I–B).
[a] Planted 2 days to 3 weeks after treatment (12 seed lots).
[b] Planted 1 year after treatment (5 seed lots).

only 50 seeds were planted in the field in each 8-foot row, and there were only three replications.

In both Series I and Series II, the seed was planted in the constant temperature rooms in soil adjusted to 55 per cent of its water-holding capacity, and contained in small metal boxes as described by Leukel.[4] Fifty beans were planted 1½ inches deep in each metal box.

In both series, all emergence counts were made when about half of the seedlings were beginning to unfold their first trifoliolate leaves.

RESULTS

The results obtained in Series I are given in tables 1, 2, and 3.

When the counts from the twelve seed lots used in Series I–A were combined, (Table 1) significant increases in stand, as compared with the untreated lots, were found from the three fungicides at 15°, 20° and 25° C., but not at 30° C. By combining the results at the four temperatures (Tables 2)

[4] Leukel, R. W. Chemical seed treatments for the control of certain diseases of sorghum. U. S. Dept. Agr. Tech. Bul. 849. 1943.

significant increases in stand were indicated from the three fungicides for most of the varieties. Exceptions were Chief (A) and Viking for Arasan; Patoka, Chief (C), and S 55–10 for Spergon; and Viking for New Improved Ceresan.

It appears from table 3 that seed treatment was more effective in increasing emergence from seed produced in the South than from that produced in Illinois. This contrast is particularly striking when the results from the variety Gibson and Nos. 42–91 and 42–53, all grown in the South, are compared with those from Chief (A), Patoka, and Viking, grown in Illinois. This is further substantiated by the fact that the seed of Chief (B), produced in Mississippi, were much lower in germination than were seed of

TABLE 2.—*Efficacy of treatments on different seed lots. Results at the four temperatures were combined*

Variety or seed lot	Average number of seedlings from 50 seeds planted							
	Series I–A 4 days after treatment with				Series I–B one year after treatment with			
	Arasan	Spergon	New Improved Ceresan	No treatment	Arasan	Spergon	New Improved Ceresan	No treatment
Chief (A)	37.85	38.65*	38.95*	35.08	——	——	——	——
Patoka	40.45**	38.33	40.40*	35.90	——	——	——	——
Viking	36.50	37.92*	36.35	33.83	——	——	——	——
Lincoln	47.50**	46.93**	48.28**	41.33	38.17**	34.34**	44.00**	27.67
Chief (B)	36.50*	37.58**	36.65*	32.83	——	——	——	——
Creole	46.50**	41.75**	46.40**	36.50	23.27**	24.08**	38.75**	13.00
Chief (C)	19.43**	14.75	21.43**	13.65	10.75	12.67*	9.67	8.50
S–100	39.30**	37.00**	39.75**	31.25	14.08**	10.67**	18.17**	5.49
S 55–10	25.10**	18.58	26.15**	15.68	——	——	——	——
Gibson	31.33**	24.83**	24.23**	15.68	——	——	——	——
42–91	36.25**	35.43**	35.08**	28.25	17.67**	15.33**	16.67**	10.09
42–53	40.00**	39.23**	38.90**	29.25	——	——	——	——

* Significant at the 5 per cent level (3.44 required for I–A, 3.79 for I–B).
** Significant at the 1 per cent level (4.52 required for I–A, 5.01 for I–B).

Chief (A) and (C), produced in Illinois, and that the greatest benefit from the seed treatment was obtained with the Mississippi-grown seed. It may be added that seed in the lot from Mississippi were more discolored and had less lustre than those in the lots from Illinois.

Emergence from seed of Mississippi-grown S 55–10 was significantly improved by treatment with Arasan at all four temperatures and with New Improved Ceresan at 20° and 25° C., but was not significantly improved by treatment with Spergon at any temperature. The differences at 25° C. are illustrated in figure 1. Apparently when seed germinate poorly or are heavily contaminated, Spergon is not so effective in improving emergence as are Arasan or New Improved Ceresan. This is apparent in seed lots of Creole and Chief (B), as well as in S 55–10.

From an analysis of variance, it was found that there was a significant difference in the reaction of the twelve seed lots (varieties) to temperature

and treatment, and that the interactions of variety × temperature, variety × treatment and temperature × treatment all had a significant F value.

The seed in Series I–A, which was planted soon after being treated, germinated better than that in Series I–B, which was planted a year after it was treated. The lower germination of the latter was especially pronounced for S 100 and 42–91 at 15° and 20° C. It is evident, however, as shown in table 3, that much better emergence was obtained from the treated stored seed than from the untreated stored seed, except for Chief (C) whose emergence was so poor that the counts were too small to show significant differences. On the whole, emergence from the treated seed exceeded that from the untreated, more in Series I–B than in Series I–A.

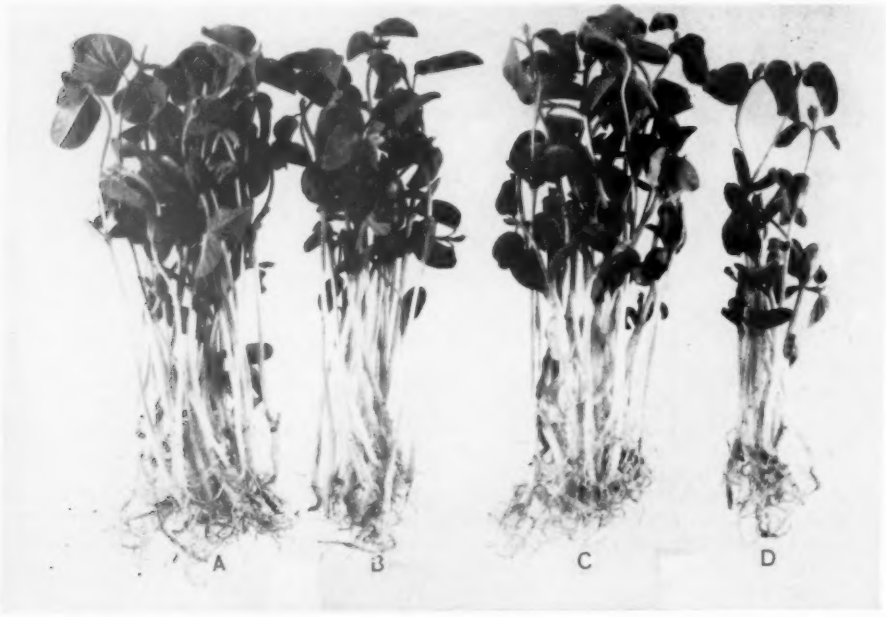

FIG. 1. Soybean seedlings of selection S 55–10 grown at 25° C. from 50 seeds treated with: A, Arasan; B, Spergon; C, New Improved Ceresan; and D, no treatment (control).

When the seedling counts from Series I–B (Table 1) were combined and subjected to the analysis of variance, significant improvement in emergence from each of the three treatments was evident at 25° and 30° C. At 20° C., significant increases in stand were obtained from treatments with Arasan and New Improved Ceresan, and at 15° C. from New Improved Ceresan only. New Improved Ceresan was the only fungicide to produce significant increases in emergence over the check at all temperatures. At 30° C., all fungicides increased stands significantly, which was not the case in Series I–A.

In Series I–B, there was a significant difference in the germination of varieties at different temperatures and treatments, and all of the interactions

TABLE 3.—*Effect of variety, source of seed, seed treatment, soil temperature, and storage upon emergence in soybeans*

Seed lot[a] and source	Treatment	Average number of seedlings emerged from 50 seeds in								Emergence in Series I–C[d] (Pct.)
		Series I–A at[b]				Series I–B at[c]				
		15° C.	20° C.	25° C.	30° C.	15° C.	20° C.	25° C.	30° C.	
Chief (A), from Edgewood, Ill.	Arasan	34.33	39.00	39.33	38.67	——	——	——	——	——
	Spergon	42.00**	35.67	38.33	38.67	——	——	——	——	——
	N. I. C.	34.33	39.33	41.33	40.67	——	——	——	——	——
	No treatment	30.33	34.67	36.67	38.67	——	——	——	——	——
Patoka, from Edgewood, Ill.	Arasan	42.00	38.33	40.67	40.67	——	——	——	——	——
	Spergon	39.00	38.33	37.00	39.00	——	——	——	——	——
	N. I. C.	40.33	40.67*	44.00*	36.67	——	——	——	——	——
	No treatment	37.00	33.00	36.67	36.67	——	——	——	——	——
Viking, from Edgewood, Ill.	Arasan	32.67	37.67	38.00	37.67	——	——	——	——	——
	Spergon	37.33**	39.00*	40.67	34.67	——	——	——	——	——
	N. I. C.	32.00	37.33	37.33	38.67	——	——	——	——	——
	No treatment	27.67	31.33	37.67	38.67	——	——	——	——	——
Lincoln, from Beltsville, Md.	Arasan	48.00*	49.33**	44.67	48.00	21.67	42.33*	45.00**	43.67**	89.75
	Spergon	45.67	47.67*	46.33*	48.00	16.67	37.00	41.00**	42.67**	87.50
	N. I. C.	49.67**	47.67*	46.67*	49.00	37.33**	46.67**	45.00**	47.00**	89.00
	No treatment	39.67	39.00	39.33	47.33	18.33	33.33	27.33	29.67	84.75
Creole, from Georgia	Arasan	45.67**	47.00**	45.00**	48.33	9.00	24.00*	32.67**	36.33**	88.25**
	Spergon	33.33	41.00	45.00**	47.67	8.67	19.67	33.33**	34.67**	88.25**
	N. I. C.	45.33**	47.33**	44.67**	48.33	23.00**	40.33**	45.33**	46.33**	92.00**
	No treatment	30.67	37.00	31.00	47.33	3.00	16.33	21.00	11.67	70.25
Chief (B), from Tunica, Miss.	Arasan	7.33	24.00**	25.67**	20.67	——	——	——	——	——
	Spergon	4.00	15.33	19.67	20.00	——	——	——	——	——
	N. I. C.	9.00	22.67**	26.33**	27.67	——	——	——	——	——
	No treatment	5.33	12.00	14.33	23.00	——	——	——	——	——
S 100, from Stoneville, Miss.	Arasan	39.33**	39.33*	40.33	38.33	0.33	10.00	27.00**	19.00**	64.25**
	Spergon	33.00**	37.33*	38.67	39.00	2.33	7.67	14.67	18.00**	66.25**
	N. I. C.	34.00**	41.33**	42.00*	41.67	3.00	17.00**	26.00**	26.67**	68.25**
	No treatment	23.67	30.33	34.33	36.67	2.00	5.33	13.33	1.33	48.75

TABLE 3—(*Continued*)

Seed lot[a] and source	Treatment	Average number of seedlings emerged from 50 seeds in Series I–A at[b] 15° C.	20° C.	25° C.	30° C.	Series I–B at[c] 15° C.	20° C.	25° C.	30° C.	Emergence in Series I–C[d] (Pct.)
S 55–10, from Tunica, Miss.	Arasan	18.67**	22.67**	28.33*	30.67*	——	——	——	——	——
	Spergon	10.33	16.00	19.67	28.33	——	——	——	——	——
	N. I. C.	12.00	29.00**	33.33**	30.33	——	——	——	——	——
	No treatment	7.67	12.00	19.33	23.67	——	——	——	——	——
Gibson, from Tunica, Miss.	Arasan	30.33**	33.00**	33.33**	28.67	——	——	——	——	——
	Spergon	16.33	25.00**	28.67**	29.33	——	——	——	——	——
	N. I. C.	16.33	21.33*	31.33**	28.00	——	——	——	——	——
	No treatment	11.00	14.33	13.67	23.67	——	——	——	——	——
42–91, from Raleigh, N. C.	Arasan	37.67**	38.33**	36.00	33.00	0.33	10.67	29.33**	30.33**	65.75**
	Spergon	31.33**	37.67**	39.00	33.67	1.33	11.00	24.33*	24.67*	71.25**
	N. I. C.	36.33**	39.00**	34.00	31.00	1.33	17.33**	29.00**	19.00	65.50**
	No treatment	18.33	27.00	32.67	35.00	0.67	7.00	15.67	17.00	54.00
42–53, from Raleigh, N. C.	Arasan	40.33**	41.33**	41.67**	36.67	——	——	——	——	75.25
	Spergon	37.00**	42.33**	42.33**	35.33	——	——	——	——	72.75
	N. I. C.	40.33**	38.33**	40.33*	36.67	——	——	——	——	69.50*
	No treatment	25.00	27.67	31.33	33.00	——	——	——	——	61.25
Chief (C), from Illinois	Arasan	32.33	34.00	38.67	41.00	3.00	12.33	15.00	12.67	——
	Spergon	37.33*	38.33*	39.67	35.00	4.00	13.67	15.67	17.33	——
	N. I. C.	35.00	39.33**	38.00	34.33	5.00	10.33	12.67	10.67	——
	No treatment	29.00	29.33	35.67	37.33	1.33	13.00	9.67	10.00	——

[a] All seed lots were harvested in 1944 except Creole which was 1943 seed.
[b] Planted 2 days to 3 weeks after treatment.
[c] Planted 1 year after treatment.
[d] Planted in field 15 months after treatment.
* Significant at the 5 per cent level (6.87 required for I–A, 7.59 for I–B, 8.00 for I–C).
** Significant at the 1 per cent level (9.04 required for I–A, 10.01 for I–B, 10.65 for I–C).

TABLE 4.—*Effect of seed discoloration, soil temperature, and seed treatment on emergence in Wood's Extra Early Yellow soybean (Series II–A)*

Class	Seed treatment	Average number of seedlings emerged from 50 seeds at		
		20° C.	25° C.	30° C.
1. At least half discolored	Arasan	19.33*	19.00**	20.00**
	Spergon	12.00	11.00	9.00
	N. I. Ceresan	13.00	14.00	4.67
	No treatment	9.67	7.33	2.33
2. Less than half discolored	Arasan	28.33**	28.33**	31.00**
	Spergon	18.67	22.67**	17.33**
	N. I. Ceresan	18.00	15.67*	5.67
	No treatment	12.33	7.33	.67
3. No discoloration	Arasan	25.67**	29.33**	22.00**
	Spergon	18.33	21.67**	11.33**
	N. I. Ceresan	21.33	22.67**	8.67**
	No treatment	14.33	4.00	1.00

* Significant at 5 per cent level (7.64 required).
** Significant at 1 per cent level (10.17 required).

had a significant F value. When the seedling counts from all temperatures were combined and analyzed for variance, those from the treated seed showed significant increases over the checks for most varieties and treatments. The only exception was Chief (C) for which Arasan and New Improved Ceresan did not improve stand significantly.

In Series I–C, planted in the field (Table 3), seedling emergence was significantly improved by all three fungicides in all five seed lots except Lincoln. The difference between the average counts of seedlings from treated and untreated seed of the other four lots was significant at the one per cent level, except in seed lot 42–53, treated with New Improved Ceresan, in which the difference was significant only at the 5 per cent level.

In Series II, in which Wood's Extra Early Yellow had been separated into three classes, it was expected that the more discolored the seed lot the lower the germination would be, and the greater the improvement from seed treatment. The data on emergence in Series II–A (Table 4) show that in general the more discolored the seed lot the lower the emergence but, con-

TABLE 5.—*Analysis of variance applied to data on emergence in Wood's Extra Early Yellow soybean combining the results from the 3 temperatures (Series II–A)*

Class	Average number of seedlings emerged from 50 seeds treated with			
	Arasan	Spergon	New Improved Ceresan	No treatment
1. At least half discolored	19.44**	10.67	7.22	6.44
2. Less than half discolored	29.22**	19.56**	13.11**	6.78
3. No discoloration	25.67**	17.11**	17.56**	6.44

* Significant at 5 per cent level (4.41 required).
** Significant at 1 per cent level (5.87 required).

trary to expectation, the less the improvement from seed treatment. In Class 1 (Table 5) in which the poorest stand was produced, Arasan was the only treatment that significantly improved emergence, and it was also the most effective treatment for the other classes. More improvement from treatment was obtained at 25° C. and 30° C. than at 20° C. (Table 6).

There was a significant difference in the germination of varieties at different temperatures, and the interactions of varieties × treatments and temperatures × treatments were significant. There was no significant difference between replications and the interaction of variety × temperature did not have a significant F value.

TABLE 6.—*Effect of fungicides at 3 temperatures on emergence in Wood's Extra Early Yellow soybeans combining the results of all seed lots in Series II–A*

Treatment	Average number of seedlings emerged from 50 seeds at		
	20° C.	25° C.	30° C.
Arasan	24.44**	25.55**	24.33**
Spergon	16.33	18.45**	12.55**
New Improved Ceresan	17.43*	17.45**	6.34*
No treatment	12.11	6.22	1.33

* Significant at 5 per cent level (4.41 required).
** Significant at 1 per cent level (5.87 required).

In Series II–B, planted in the field, there was no significant difference in emergence between the controls that were left untreated and the treated seed lots, and there was no significant difference between treatments.

SUMMARY

Temperature had a marked effect on the response of several lots of soybeans to seed treatment. Increases in germination from seed treatment were obtained more often at 25° C. than at any other temperature.

All three fungicides used usually improved emergence from the different seed lots, but Arasan improved it more frequently than either Spergon or New Improved Ceresan.

Seed obtained from Georgia, Mississippi, North Carolina, Maryland, and Virginia appeared to be benefited more by seed treatment than did seed produced in Illinois.

Soybean seed treated and then stored for one year gave a relatively greater increase in emergence over the check than did the treated seed planted soon after treatment, but total emergence was less after storage.

The moderately discolored and the non-discolored seed lots of Wood's Extra Early Yellow soybeans showed greater response to seed treatment than did the badly discolored seed lot, when grown in the constant temperature rooms. When grown in the field, however, there was no significant difference between the reactions of the three lots of seed.

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THE RELATION OF SIX GROUPS OF FUNGI TO SEEDLING DISEASES OF SUGAR BEETS IN MONTANA[1]

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INTRODUCTION

Seedling diseases or root rots are of considerable importance in the growing of sugar beets, especially in the heavy irrigated soils in Montana. Numerous investigations have been made on these diseases to determine their nature and control in Montana (1, 2, 5) and elsewhere (3). Pathogenic fungi have been isolated many times, and have been classified in the following genera: *Phoma, Macrosporium, Fusarium, Rhizoctonia, Aphanomyces,* and *Pythium.*

MATERIALS AND METHODS

For isolating *Phoma, Macrosporium, Fusarium,* and *Rhizoctonia* potato-dextrose agar (20 gm. agar, 20 gm. dextrose, and 200 gm. sliced potatoes per liter of water) was used. Diseased beet seedlings were thoroughly washed in tap water, the diseased portions of the roots were cut off and washed in several changes of sterilized water, and then small portions of the diseased roots were imbedded in potato-dextrose agar in Petri dishes. Potato-dextrose agar is a very rich medium and permits luxuriant growth of saprophytic and weakly pathogenic organisms, therefore many of them were isolated by this method.

Cornmeal agar was used for isolating Phycomycetous fungi, suspected of being responsible for seedling diseases of beets. Diseased beet seedlings were thoroughly washed in tap water and the diseased portions of the roots were cut off and placed in sterile Petri dishes filled with sterile water. The beet roots were kept in the Petri dishes for about 24 hours, during which period the sterile water was changed several times. The tissue culture isolations were made on cornmeal agar (15 gm. agar, 20 gm. cornmeal per liter of distilled water).

As soon as the fungal growth appeared it was examined, and very often other kinds of fungi and bacteria occurred with Phycomycetous fungi. The separation of Phycomycetous fungi from other kinds of fungi was made by successive transfers of high dilutions of mixed cultures to cornmeal agar. To separate Phycomycetous fungi from bacteria the following technic was used: High dilutions of organisms were made on cornmeal agar and as soon as fungus colonies appeared, the plates were put in the refrigerator at 5° C. where the fungi continued to grow but bacterial growth was suppressed. Transfers from the edges of the colonies gave fungal growth free of contaminating bacteria. Kauffman's beef-gelatin (4) was also used in separating Phycomycetous fungi from bacteria and the other fungi.

[1] Contribution from Montana State College, Agricultural Experiment Station, Paper No. 191, Journal Series.

One hundred and eight isolates of different fungi were accumulated, and 85 of these were tested for pathogenicity in the greenhouse.

Soil for the pathogenicity studies was obtained from the Huntley Branch Station, Huntley, Montana, and was disinfected with chloropicrin. This soil can be considered typical of the majority of beet soils of Eastern Montana. Duplicate jars of soil were inoculated with each of the cultures of *Phoma, Macrosporium, Fusarium,* or *Rhizoctonia* grown on steamed rice. Two or more jars of soil were not inoculated and were used as checks for each group of organisms tested. Fifteen sugar-beet seedballs treated with New Improved Ceresan (1 oz. for 20 lb. seed) were planted in each jar of soil.

In testing the pathogenicity of reisolated cultures of these fungi, the soil was sterilized in the autoclave and the inoculum was grown in Petri dishes on potato-dextrose agar.

The pathogenicity of the original and reisolated cultures of *Aphanomyces* and *Pythium* was tested in soil sterilized in an autoclave. Two clay pots of soil were inoculated with each of the isolates grown in Petri dishes on corn-meal agar. Two uninoculated pots of soil for each group of organisms were used as checks. Ten sugar-beet seedballs treated with New Improved Ceresan were planted in each pot.

The beets were grown in all these tests for about one month, and healthy and diseased seedlings were recorded at regular intervals. At the end of this period the beet seedlings were dug, washed, and final readings were made.

EXPERIMENTAL RESULTS

In these tests thirteen cultures of *Phoma,* ten cultures of *Macrosporium,* forty-seven cultures of *Fusarium,* five cultures of *Rhizoctonia,* eight cultures of *Aphanomyces cochlioides,* and two cultures of *Pythium* were tested for pathogenicity (Table 1).

The beets in the check pots used for plants inoculated with cultures of *Phoma, Macrosporium, Aphanomyces cochlioides,* and *Pythium* remained healthy. A few of the check plants became diseased in the *Fusarium* and *Rhizoctonia* series. This was probably due to accidental contamination or to the partial sterilization of the soil with chloropicrin. In all of the series where steam sterilization of the soil was used the checks remained healthy.

Four reisolated cultures of *Phoma, Macrosporium,* and *Fusarium,* one of *Rhizoctonia,* eight of *Aphanomyces cochlioides,* and two of *Pythium* were tested for pathogenicity (Table 1). The beets grown in the checks for these tests remained healthy.

The number of emerged plants in all inoculated and check pots for all isolated and reisolated fungi was about the same with the exception of those inoculated with *Phoma,* which indicated that there was very little pre-emergence killing of the plants. Beets grown in pots inoculated with *Phoma* had a much lower stand than in the checks, indicating that there probably was considerable pre-emergence killing.

At the time of harvest many diseased beet seedlings infected with any of these fungi showed a tendency to recover by sloughing off the infected cortical layer of the hypocotyl.

Symptoms of Disease and Degree of Pathogenicity of the Fungi Used in These Tests

Species of Phoma

All cultures of *Phoma* were highly pathogenic to sugar-beet seedlings. The average number of diseased seedlings was 89.1 per cent, but this percentage varied from 33.4 to 100.0 for individual cultures (Table 1). The degree of infection was variable, some beets were infected severely and others only slightly. A culture of *Phoma betae* (Oud) Fr. obtained from the American Type Culture Collection, was also tested for pathogenicity in a

TABLE 1.—*Pathogenicity test of six groups of fungi on sugar-beet seedlings*

Fungus	Original isolates	Reisolates	Percentage[a] of seedlings diseased			Pathogenicity rating
			Min.	Max.	Av.	
	Number	*Number*				
Phoma	13	.....	33.4	100.0	89.1	Medium
Do	.....	4	2.2	71.3	25.3	Variable
Macrosporium	10	.....	6.4	40.9	21.8	Slight
Do	.....	4	0.0	6.6	4.1	Slight
Fusarium	47	.....	3.8	95.5	14.9	Slight
Do	.....	4	tr.	9.4	4.0	Slight
Rhizoctonia	5	.....	8.0	28.5	18.9	Slight
Do	.....	1	.....	.....	20.0	Slight
Aphanomyces cochlioides	8	.....	95.0	100.0	99.4	Severe
Do	.....	8	93.4	100.0	98.6	Severe
Pythium	2	.....	82.3	87.3	84.8	Medium
Do	.....	2	5.3	12.4	8.8	Slight

[a] Minimum and maximum of diseased beet seedlings produced by any one of the cultures. Average of diseased beet seedlings produced by all cultures.

comparison with isolations in Montana. This culture proved to be only slightly pathogenic to sugar-beet seedlings.

The total stand of beet seedlings varied considerably for the different cultures tested. On young infected plants that were not killed before emergence the symptoms of disease usually appeared at time of emergence or soon afterwards. The leaves of severely infected seedlings wilted slightly and usually drooped to the ground. These were somewhat dark-green to bluish instead of the light-green of healthy seedlings. These infected plants usually had dark-brown discolorations of the hypocotyls and some of them were girdled. None of the emerged plants died before harvest. Besides this severe type of infection, numerous plants had slight infection of the underground parts of hypocotyls and roots which could not be detected before harvest. These infections were either brownish discolorations or

lesions. Except for a slight stunting, such seedlings externally appeared to be normal plants.

Four reisolated cultures of *Phoma* were tested for pathogenicity (Table 1). One was very slightly, two were slightly, and one was severely pathogenic to sugar-beet seedlings. The average number of diseased seedlings was 25.3 per cent, but this percentage varied from 2.2 to 71.3 for individual cultures. The symptoms and the development of the disease were similar to those produced by the original cultures.

In conclusion, all isolated and reisolated cultures of *Phoma* proved to be pathogenic to beets and the degree of pathogenicity varied for different cultures.

Species of Macrosporium

Only four cultures of *Macrosporium* produced visible symptoms of disease before the seedlings were dug (Table 1). These diseased plants had yellowing and slight browning of their hypocotyls and some of them were girdled. At harvest time diseased seedlings were found in all sets and their average was 21.8 per cent, but this percentage varied from 6.4 to 40.9 for individual cultures. All diseased roots had slight yellowish-brown discoloration and this was either uniformly present all over the roots or was confined only to certain localized areas. Those plants which had discoloration above the ground were slightly stunted in growth, the remaining plants appeared to be normal. None of the infected plants died before harvest.

Four reisolated cultures of *Macrosporium* were tested for pathogenicity (Table 1). One culture was nonpathogenic and the remaining three only slightly pathogenic. The average number of diseased seedlings was 4.1 per cent, but this percentage varied from 0.0 to 6.6 for individual cultures. The symptoms and the development of the disease were similar to those produced by the original cultures.

The degree of infection for all isolated and reisolated cultures of *Macrosporium* was slight and none of the infected beets died before harvest.

In conclusion, all isolated and practically all reisolated cultures of *Macrosporium* proved to be only slightly pathogenic to beets, and the degree of pathogenicity varied for the different cultures.

Species of Fusarium

All cultures of *Fusarium* were not tested for pathogenicity at once but in several separate tests and all were found to be pathogenic to sugar-beet seedlings. The average number of diseased seedlings was 14.9 per cent, but this percentage varied from 3.8 to 95.5 for individual cultures (Table 1). Diseased seedlings usually showed only light-brown discoloration of their roots. With a few cultures, however, the roots had dark-brown to black discoloration. Also, a few cultures developed a light-brown or black discoloration at the place of seed attachment. One of the cultures produced a dark-brown discoloration of the hypocotyl. Although the infection in

general was slight, in a few jars several infected beets died at the beginning of the experiment.

All four reisolated cultures of *Fusarium* which were tested for pathogenicity (Table 1) were slightly pathogenic to sugar-beet seedlings. The average number of diseased seedlings was 4.0 per cent, but this percentage varied from a trace to 9.4 for individual cultures. The symptoms and the development of the disease were similar to those produced by the original cultures. None of the infected plants died before harvest. In general, the isolated and reisolated cultures of *Fusarium* proved to be slightly pathogenic to beet seedlings, although the percentage of infection was high for some of the cultures.

Species of Rhizoctonia

All cultures of *Rhizoctonia* were pathogenic to sugar-beet seedlings. The average number of diseased seedlings was 18.9 per cent, but this percentage varied from 8.0 to 28.5 for individual cultures (Table 1). All infected plants had a light brown discoloration of the lower parts of their roots. The infection was slight and not a single infected beet died before harvest.

One reisolated culture of *Rhizoctonia* was tested for pathogenicity (Table 1). Twenty per cent of seedlings were diseased and they had dark lesions on all parts of the roots. Some of the roots were girdled and the lower part of the root was lost. Many of the seedlings had remnants of dark infected regions of the hypocotyls immediately below the crown and traces of infection on their roots. Many of this kind of beet were classified as healthy. The degree of infection was slight to medium and several infected beets died before harvest. The symptoms of disease as they are described for reisolated cultures are usually associated with *Rhizoctonia* infection of beet seedlings in field.

In conclusion, all isolated and reisolated cultures of *Rhizoctonia* proved to be pathogenic to sugar-beet seedlings. The degree of pathogenicity varied with different cultures.

Aphanomyces cochlioides Drechs.

Practically all beets inoculated with eight cultures of *Aphanomyces cochlioides* became diseased, the average number of diseased seedlings being 99.4 per cent, but this percentage varied from 95.0 to 100.0 for individual cultures (Table 1). The type of disease produced on sugar-beet seedlings was rather uniform and resembled very closely the "black-root" type of seedling disease occurring in the field. Hypocotyls of practically all diseased seedlings were completely discolored, dark-brown to black, and only a few diseased plants still had remains of healthy hypocotyl tissue. Hypocotyls always had more pronounced discolorations than the roots. This indicates that the disease probably starts either at the surface of the ground or only slightly below it and progresses more upward than downward. The bases of some of the lower leaves were also slightly affected, showing a slight

dark-brown discoloration, but all the leaves remained green and turgid. The diseased plants, stunted in growth and with their stems dark-brown to black and dry, retained their green leaves and turgidity for a long time. There was considerable mortality of the diseased plants before harvest, although on an average more than 50 per cent survived. Infection in most cases was severe.

Practically all beets inoculated with eight reisolated cultures of *Aphanomyces cochlioides* became diseased. The average number of diseased seedlings was 98.6 per cent, but this percentage varied from 93.4 to 100.0 for individual cultures (Table 1). The progress of the disease and the symptoms were exactly the same as for the original cultures.

In conclusion, all isolated and reisolated cultures of *Aphanomyces cochlioides* proved to be highly pathogenic to sugar-beet seedlings.

Species of Pythium

Both cultures of *Pythium* developed a considerable amount of disease. The average number of diseased seedlings was 84.8 per cent, but this percentage varied from 82.3 to 87.3 for individual cultures (Table 1).

During the progress of the disease, before any visible discoloration of the hypocotyls was noticeable, the leaves of diseased beets wilted and drooped. Later, diseased plants became stunted and chlorotic (lemon color) and eventually wilted, and many of them died. Most of the seedlings had a light-brown discoloration of their roots at harvest time. Less than half of the diseased seedlings died before harvest, and the remainder had only a mild degree of infection. Apparently the infection occurred either at the surface of the ground or below it and the injury was confined almost entirely to the roots.

At the time the reisolated cultures of *Pythium* were tested for pathogenicity only a few plants became diseased. The average number of diseased seedlings was 8.8 per cent, but this percentage varied from 5.3 to 12.4 for individual cultures (Table 1). In general, the infection was slight and the symptoms of diseased seedlings closely resembled those inoculated with the original cultures, except that very little yellow discoloration was produced on the hypocotyls of diseased seedlings. Only one diseased plant died before harvest.

In conclusion, the original isolates of *Pythium* were more pathogenic to beet seedlings than reisolated cultures.

DISCUSSION

Results are presented in which beet seedlings were artificially inoculated with six different groups of fungi.

In evaluating these organisms as possible causal agents of seedling diseases of sugar-beets it appears that the symptoms of diseased seedlings, artificially inoculated with *Aphanomyces cochlioides* resembled most closely the majority of beets naturally infected in fields in Montana.

The symptoms of diseased plants artificially inoculated with *Phoma* resembled to some extent the ones infected with *Aphanomyces cochlioides*, except that the leaves of *Phoma*-infected plants showed some wilting; while those infected with *Aphanomyces cochlioides* showed practically none. Also there was a considerable amount of pre-emergence killing of the plants inoculated with *Phoma* and practically none in those inoculated with *Aphanomyces cochlioides*. Undoubtedly every year some beet seedlings are infected with *Phoma* in the field, although the percentage probably is not great.

The symptoms of diseased seedlings artificially inoculated with *Pythium* are typical of damping-off disease and were not observed to any great extent in diseased beets under field conditions. It is believed that *Pythium* has only a minor significance in seedling diseases of sugar beets in Montana.

The pathological symptoms of beets infected by *Macrosporium* and *Fusarium* are not very distinct and for this reason it is difficult under field conditions to diagnose the causal factor of a disease on the basis of the symptoms alone. These symptoms may be confused with the early stages of disease produced by either *Aphanomyces cochlioides* or *Phoma*.

Under the conditions of artificial inoculation, which are very conducive to infection, fungi like *Fusarium* and *Macrosporium* proved to be only slightly pathogenic to beet seedlings. Under competitive field conditions their pathogenicity will be even less. They probably act only as secondary invaders.

The symptoms of diseased seedlings artificially inoculated with *Rhizoctonia* are typical for this disease and beets with this type of symptoms always could be found in a small number in the field.

It appears that seedling diseases of sugar beets from the standpoint of the organisms involved are complex in nature, but under Montana conditions, *Aphanomyces cochlioides* is responsible for more seedling diseases of sugar beets than any other of the above mentioned fungi.

A high percentage of artificially inoculated beets survived. This condition is usually observed in the field. If diseased beets survive until they are approximately in a 6th leaf stage, their recovery usually is assured. Since the number of young beet seedlings present in the field before thinning is always several times greater than that which is required for a proper stand, it appears that even with a bad case of seedling disease it is possible to obtain more or less a normal stand by using delayed, selective thinning. However, the damage to the crop usually results not so much from the reduced stand, as from the retarded growth of plants of low vigor. It is generally true that with a comparatively good stand of beets in the field, in which there was an abundance of seedling disease in the spring, the yields are usually poor because of small beets.

In Montana, seedling diseases of beets can be effectively controlled by improving the physical conditions of the soil and by providing sufficient and

balanced fertilization (1, 2, 5). This usually results in a good stand, rapid and vigorous development of young beets, and high yields.

SUMMARY

1. Numerous isolations of different fungi were made from diseased sugar-beet seedlings in Montana.

2. Pathogenicity of 13 isolated cultures of *Phoma*, 10 of *Macrosporium*, 47 of *Fusarium*, five of *Rhizoctonia*, eight of *Aphanomyces cochlioides*, and two of *Pythium* was tested on sugar-beet seedlings.

3. *Fusarium*, *Macrosporium*, and *Rhizoctonia* were only slightly pathogenic, *Phoma* and *Pythium* were moderately so, and *Aphanomyces cochlioides* was the most pathogenic on sugar-beet seedlings.

4. The symptoms produced by *Aphanomyces cochlioides* on artificially inoculated beets resemble most closely those manifested by the diseased seedlings in the field.

5. Although seedling diseases of sugar beets from the standpoint of the organisms involved are complex in nature, it is believed that *Aphanomyces cochlioides* is responsible for most of the disease in Montana.

6. It is evident that some of the fungi studied are only weak parasites, and possibly they act as secondary invaders.

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KENTUCKY WONDER BEAN PLANTS AS HOSTS FOR MEASURING SOUTHERN BEAN MOSAIC VIRUS ACTIVITY[1]

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(Accepted for publication November 7, 1947)

The Early Golden Cluster variety of garden bean (*Phaseolus vulgaris* L.) was first used for virus activity measurements in 1930 (6). Since then it frequently has been applied in studies on a number of different viruses. A recently described method for estimating the relative activity of Southern bean mosaic virus (*Marmor laesiofaciens* Z. et H.) with errors usually less than 10 per cent makes use of the necrotic local lesions produced by the virus on Early Golden Cluster bean plants (7). Unfortunately, seed of this variety has not been obtainable from commercial houses during the past two years. It has therefore been necessary to make use of another variety of *P. vulgaris* in carrying out activity studies on Southern bean mosaic virus. This paper describes the use of Kentucky Wonder bean plants for such activity measurements.

Most varieties of *Phaseolus vulgaris* respond to infection with Southern bean mosaic virus either by the production of necrotic local lesions or of systemic mottling (10). Symptom expression is controlled by a single pair of allelomorphic factors, localization being dominant to systemic mottling (9). Zaumeyer and Harter (10) reported that the Kentucky Wonder varieties carried the dominant gene for the local lesion response. These varieties were therefore tested for their suitability for activity measurements of Southern bean mosaic virus.

Plants were grown 6 to a pot in 4-inch pots in composted soil in a greenhouse and thinned out to 3 before being used. When the primary leaves reached about three-quarters their mature size they were inoculated with one or another of various dilutions of Southern bean mosaic virus. The virus used for inoculation was obtained from three-weeks infected plants of the Bountiful variety by grinding in a food chopper and expressing the sap through a layer of cheesecloth. Dilutions of the sap were made in appropriate volumes of 0.1 M potassium phosphate buffer at pH 7.

Both Kentucky Wonder Wax and Kentucky Wonder Green Pod leaves developed typical necrotic lesions after inoculation. Table 1 summarizes the numbers of lesions produced on 36 leaves of each of the two varieties with each of 6 concentrations of Southern bean mosaic virus.

The nature of the virus dilution curve has been considered by several workers (1, 3, 5, 8). Recently, Lauffer and Price (4) reviewed the literature on this subject and reached the conclusion that the most tenable hypothesis for explaining the character of the curve is the simple hypothesis that the probability of obtaining infection is related to the probability of find-

[1] Aided by a grant from the National Foundation for Infantile Paralysis, Inc.

ing a single infectious unit in an elemental volume of solution that comes into intimate contact with a susceptible region of the host surface. If y represents the number of local lesions produced with a dilution x of a solution containing n particles per cc., v an elemental volume coming into contact with a susceptible region of the host surface, and N the number of susceptible regions, then

$$y = N(1 - e^{-vnx}) \tag{1}$$

This equation was applied to virus dilution curve data by Youden, Beale, and Guthrie (8) and independently by Bald (1). If the particles

TABLE 1.—*Total numbers of lesions produced on 36 leaves of Kentucky Wonder Wax and Kentucky Wonder Green Pod bean plants with each of 6 concentrations of Southern bean mosaic virus*

Log. virus concentration	Kentucky Wonder Wax	Kentucky Wonder Green Pod
0	50,400	39,600
−1	31,320	30,960
−2	25,920	14,400
−3	7,920	2,160
−4	1,980	324
−5	144	36

in the solution are aggregated into groups of 2 or more and if b is taken as the number of aggregates and K is a constant, then

$$y = N(1 - e^{-vbx}) \tag{2}$$

where

$$vbx = \frac{\sqrt{1 + 4Kvnx} - 1}{2\ K} \tag{2a}$$

Equation 2 was developed by Bald (2) when it was observed that many sets of virus dilution data failed to fit equation 1 precisely.

The data of table 1 were fitted to the curve of equation 2 with the following constants: K was 2.0 for both varieties, N was 45,000 for the wax variety and 35,000 for the green pod, while vn was 300 for the wax and 95 for the green pod. In figure 1, log (y/N) is plotted as a function of log (vnx). It will be seen from this figure that the experimental data give an excellent fit to the theoretical curve.

The region of the curve most suitable for virus activity measurements is that between log (vnx) = −0.25 and log (vnx) = −2.0 (7). This means that for Kentucky Wonder Wax log (x) should be between −2.75 and −4.5 and for Kentucky Wonder Green Pod it should be between −2.25 and −4.0. Since both N and vn can be expected to vary from experiment to experiment, due to variation in growing conditions and age of host plants, these limits are also expected to vary. It has been the general practice in this laboratory to prepare the standard virus preparation from freshly expressed sap of diseased Bountiful bean plants at dilutions of 10^{-3} and 10^{-4}. With few exceptions these concentrations can be expected to lie along the curve at

the most favorable points for activity measurements. It can be seen from figure 1 that over this range the log of the number of lesions produced is very nearly a linear function of the log of the virus concentration.

In the experiments summarized in table 1 and figure 1, the same virus preparation was used in inoculating both types of plants. Hence n, the number of virus particles per cc., must have been the same in both dilution series. This means that v, the elemental volume of solution coming into contact with a susceptible region of the leaf surface, was about 3 times as great for the wax variety as for the green pod. From this it can be reasoned that the susceptible area on the wax type is about 3 times as large as that on

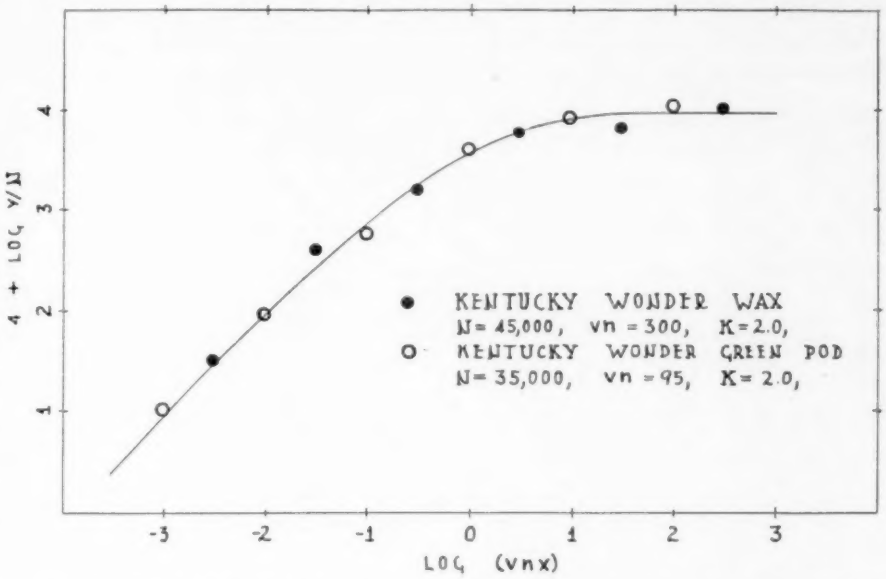

FIG. 1. Graph of Southern bean mosaic virus dilution curve when Kentucky Wonder varieties of bean were used as host plants.

the green pod. Since N was 45,000 for the wax bean and 35,000 for the green pod, and since an equal number of leaves were used in both cases, there were apparently about 28 per cent more susceptible areas on the wax than on the green pod variety.

It is of interest that while the two virus infection series vary with respect to N and v, factors contributed by the host, they do not vary with respect to K. This lack of variation with respect to K is to be expected if K measures the degree of aggregation of virus particles in the virus solution, as was assumed in the derivation of equation 2a. It would be of value to carry out additional dilution curve studies with different preparations of virus to determine whether or not K is a constant for each virus preparation.

Both Kentucky Wonder Wax and Kentucky Wonder Green Pod varieties have been used in this laboratory to measure activities of Southern bean mosaic virus preparations. While there are no data available for determining

precisely the accuracy of these measurements, it appears from general considerations that they are as accurate, if not more accurate, than those involving the Early Golden Cluster variety as a host. In table 2 are presented data from an experiment designed to measure the activity of a virus sample which was expected to have an activity slightly less than that of the standard against which it was tested. When the figures in table 2 were converted to logarithms, the logarithm of the relative activity of the unknown in terms of the standard and its standard error were calculated according to the method previously described (7). The calculated value is -0.182, indicating a relative activity of 66 per cent. The calculated standard error of the log is 0.053. The standard error is of the same order as that found previously when the Early Golden Cluster variety was used as the test plant (7). The standard errors calculated from similar experi-

TABLE 2.—*Numbers of lesions produced on 3 half leaves of Kentucky Wonder Green Pod bean plants with different concentrations of Southern bean mosaic virus*[a]

Pot. No.	S_1	U_1	S_2	U_2
	(L)	(R)	(L)	(R)
1	126	164	393	502
2	105	146	306	267
3	117	147	326	373
4	72	112	270	255
	(R)	(L)	(R)	(L)
5	173	69	463	303
6	102	49	297	306
7	213	63	300	187
8	125	71	343	268

[a] S and U refer to the standard and unknown virus preparations, L and R refer to the left and right halves of the test plant leaves.

ments performed during the last 18 months have been of the same order. From the results mentioned above it can be concluded that either Kentucky Wonder Wax or Kentucky Wonder Green Pod can serve as a suitable substitute for the Early Golden Cluster variety in measuring the activity of Southern bean mosaic virus.

SUMMARY

The numbers of necrotic lesions produced on both Kentucky Wonder Wax and Kentucky Wonder Green Pod bean plants, when inoculated with a series of dilutions of Southern bean mosaic virus, were found to follow an equation derived on the assumption that the virus particles are aggregated and that the chance of obtaining infection is related to the chance of finding a single infectious aggregate, or particle, in a unit volume of inoculum. It is a characteristic of this equation that over a certain range the log of the number of lesions is very nearly a linear function of the log of the virus concentration. It follows that the Kentucky Wonder varieties of bean are suitable test plants for use in the method of measuring Southern bean mosaic

virus activity that involves comparison of two dilutions of the unknown virus preparation with two dilutions of a standard. Experimental data obtained over a period of 18 months, an example of which is given in the text, bear out this conclusion.

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COMPARISON OF TWO METHODS OF PELLETING ONION SEED IN THE CONTROL OF SMUT

M. B. LINN[1] AND A. G. NEWHALL[2]

(Accepted for publication November 12, 1947)

A method for pelleting vegetable and other kinds of seed has been developed by the Farmers and Manufacturers Beet Sugar Association, Saginaw, Michigan in cooperation with the Dow Chemical Company, Midland, Michigan. The primary purpose is to add bulk to light seed and to make all individual seeds uniform in size in order to facilitate precision sowing with mechanical equipment. Briefly, the process involves placing the seed in a revolving, spherical-shaped pan, then alternately wetting with a binder (methyl cellulose solution) and dusting with feldspar and other materials until round pellets of the desired dimensions have been formed. Thus fungicides, insecticides, hormones, fertilizers and the like can be built into the "pill" and planted with the seed.

Another method of pelleting seed described by the junior author[3,4,5] in 1944 was developed in New York as a replacement for the laborious formaldehyde drip method of controlling onion smut. It consists of moistening the seed by stirring with a 5 per cent solution of methyl cellulose and then coating with an equal weight of an organic sulfur fungicide, Arasan or Tersan, by agitation for 5 minutes in an electric paint can shaker, or, as the senior author has found satisfactory, by rolling in a barrel or churn. The feldspar excipient is not used in the New York method and the sticker solution is less concentrated.

The pellets resulting from the two methods are somewhat different, as seen in figure 1. The use of feldspar and of repeated applications of a more concentrated sticker solution results in a comparatively large, hard, uniformly spherical pellet which on first sight would appear to be very desirable. In the New York method the pellets come out, not quite spherical, and more or less mealy rather than hard or brittle. However, the New York method is less expensive than the Michigan method and better adapted to the needs of a temporary portable system of custom treatment or of individual farm treatment of seed. Experiments conducted in Illinois and New York comparing Early Yellow Globe onion seed treated by each method were carried on by the writers in 1945 with the idea of evaluating the treatments from the standpoint of smut control, stand of seedlings, and ease of sowing in the conventional type seeders now in use.

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[3] Newhall, A. G. Studies on vegetable seed treatments in 1943. U. S. Dept. Agr., Plant Dis. Reptr. Supl. **145**: 1–97, Onion Seed Treatments, p. 39–45. 1944.

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[5] ———. Pelleting onion seeds with fungicides. Farm Research **11**: 18, 20, 1945.

Two one-pound samples were pelleted with Arasan and Tersan respectively through the courtesy of Mr. Arthur A. Schupp and Dr. Phelps Vogelsang at Saginaw, by the Beet Sugar Association feldspar method hereafter designated the Michigan method while two more were treated at Cornell by the junior author, one each with Arasan and Tersan. The rate was a pound of fungicide to a pound of seed. One pound was left untreated. The Michigan treated seed was several times larger due to the amount of sticker and excipient employed, in addition to the pound of fungicide.

One portion of each of the above 5 samples of seed was sown in Cook County, Illinois, in a field heavily infested with smut. Hole 20 of the No.

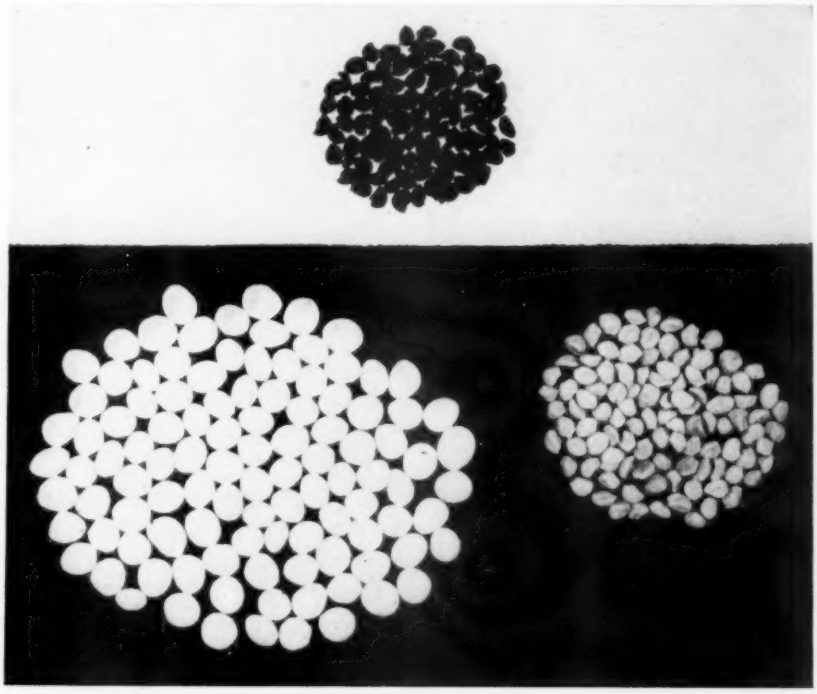

FIG. 1. Untreated onion seed (top), compared with same number pelleted by the Michigan feldspar method (lower left), and by the New York method (lower right).

300 Planet Jr. drill had to be used in sowing the Michigan pelleted seed, hole 10 for the New York pelleted seed, and hole 8 for the untreated in order to sow approximately the same number of seeds per foot. In New York four plantings were made. Two were in the greenhouse and consisted of replicate samples of 100 seeds each. A similar one was made in the field, and a larger test, also in the field, employed an Iron King single row drill on several rows 300 feet long. Deep sowing in Illinois was coupled with excessive rainfall and, therefore, delayed emergence resulted in poor smut control. In the first New York greenhouse test six replications of 100 seeds were sown and although the differences in smut control were slightly in

favor of the New York method over the Michigan method they are not significant at the 5 per cent level. All are significantly better than the check. In the second greenhouse test made in New York an excess of moisture was purposely applied with the result that considerable late infection took place giving high smut counts. In the small New York field test there was so little smut that the significant benefits over the check from all treatments, replicated 5 times, can be attributed chiefly to control of damping off, there being no significant differences in smut control. Maggot injury was so severe in the fourth New York unreplicated field test that no smut or stand counts were made, but the effect of the rotating agitator of the drill on the

TABLE 1.—*Comparison of Michigan feldspar-pelleted onion seed with New York-pelleted seed in the control of smut*

Location of test	Kind of plots	Fungicide	Pelleting process	No. plants emerged and counted	Ave. percentage smutted seedlings[a]
New York	Green-house, 1	Arasan	Michigan	95	10.6
			New York	92	9.6
		Tersan	Michigan	84	14.3
			New York	95	8.4
		None	None	72	51.5
	Green-house, 2	Arasan	Michigan	89	23.0
			New York	82	23.0
		Tersan	Michigan	76	22.0
			New York	81	19.0
		None	None	73	53.0
	Field	Arasan	Michigan	70	3.1
			New York	69	1.4
		Tersan	Michigan	67	0.5
			New York	70	2.7
		None	None	55	7.0
Illinois	Field	Arasan	Michigan	100	70.0
			New York	100	40.0
		Tersan	Michigan	100	78.0
			New York	100	52.0
		None	None	100	94.0

[a] In the New York tests, this represents the average of five replicated plots in the greenhouse and five in the field using 100 seeds in each plot. Plots were unreplicated in the two field tests.

stability of the pellets and seeding rate was closely observed. The results of these tests are summarized in table 1.

It would appear from all of the tests that the Michigan method employing feldspar did not give superior smut control nor better stands than the less expensive New York method, in fact what little difference there was seemed to be slightly in favor of the latter. In these and other tests both writers observed a considerable amount of unavoidable cracking of the feldspar pellets when sown with seeders having metal or wooden based agitators. The longer the rows the more of this occurred and in the Illinois test the poorer control of smut may have been due in part to this destruction of the

pellet. The seeding rate was believed to have slowly increased in one New York trial as time went on and more seeds were freed from their coating.

Since cracking of the pellets was thought to be due in part at least to interfacial friction, tests were run in Illinois to see if this trouble could be corrected by treating pellets with graphite. A fine lubricating graphite was mixed with Michigan feldspar-Tersan pelleted seed at the rate of 1½ ounces to 60 pounds of pellets, which seemed to be the maximum that could be retained. Although the graphite tended to reduce friction a little, the benefit was rather slight as indicated in table 2 and the cracking of pellets and liberation of bare unprotected seeds was not eliminated. Experiments in New York by the junior author likewise resulted in the rejection of graphite for expediting the flow of onion seed mixed with dry Arasan.

TABLE 2.—*Effect of graphite on seeding rate of Michigan feldspar-pelleted onion seed*

Treatment	Number of pellets sown per foot							
	Seeder plate hole number							
	10	12	14	16	18	20	22	24
Graphite	2.6	4.1	5.7	6.1	7.5	12.8[a]	15.3	21.3
No graphite	2.3	3.2	4.4	5.6	7.0	9.3	12.8	19.8

[a] This rate is approximately equivalent to that obtained with New York pelleted seed using hole 11 and with nonpelleted seed using hole 9.

The conclusions reached are that when seed drills are available for onion growers which can handle the tough hard pellets, turned out by the Michigan feldspar method, without cracking, splitting, and crushing a large proportion of them, then the advantages of more even spacing of seeds in the ground may help offset the added expense of treatment by the Michigan method, but until then the advantages from the standpoint of smut control, economy, and even stands are somewhat in favor of the New York method of pelleting.

Both methods may at times result in two or even three seeds occurring in one pellet which can affect yield records. Counts of the samples used in these experiments revealed a greater number of doubles in the Michigan than in the New York pelleted seed (11 per cent vs. 2 per cent) which may be due to the fact that the total amount of seed was small and more difficult to treat perfectly in the Michigan apparatus.

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FIELD STUDIES ON SPREAD OF THE MILD STREAK DISEASE OF BLACK RASPBERRIES

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(Accepted for publication November 12, 1947)

Mild streak, a disease apparently of virus nature, is the most serious trouble affecting black raspberries in Maryland. This disease was first observed in Maryland about 1935 and since that time has increased to such an extent that nearly all plantings in the State are affected.

Loss from mild streak is due to the effect on the fruit. Fruit symptoms vary to some extent in different varieties and there are also seasonal variations. However, typical fruit symptoms consist of loss of gloss and a pitting or shrinking of the individual drupelets. This results in undersized, dull, seedy, unattractive fruit of poor flavor.

Discolored streaks of varying intensity can usually be found on the lower portion of young canes affected with mild streak. This symptom varies greatly with variety and season but generally consists of elongated, water-soaked markings which later in the season become more clearly defined and somewhat purplish. However, in many instances the streaks are so faint as to be barely discernible. Fruit and cane symptoms are shown in figure 1.

Mild streak was first described as being distinct from severe streak by Rankin (6) in 1931, but he had apparently been familiar with this disease for several years previous. In 1932 Cooley (3) presented a complete description of the symptoms of mild streak. Since this time various other workers have presented descriptions of mild streak (1, 2, 4, 5, 7, 8, 9, and 10). Rankin (6) had previously shown that plants within ten feet of mild streak infected plants were much more apt to become infected than those further away. Rankin also proved that mild streak spread more rapidly in the variety Ohio than in Plum Farmer or Cumberland. Cooley (3) stated that the rate of spread is usually slow; about doubling each season. However, in vigorous plantings, the rate was more rapid. Woods and Haut (10) reported the spread of mild streak to be rather rapid up to 20 feet but slower up to 200 feet.

To obtain information leading to possible control measures, a study of mild streak under field conditions was begun. In the spring of 1941 about 2,500 plants of the Cumberland black raspberry were obtained from the Ohio Small Fruit Improvement Association. These plants showed no symptoms of mild streak during the first growing season. The field in which these plants were set had not been previously planted to raspberries but the north side was bordered by a hedge row containing wild black raspberries and blackberries and was about 200 yards from several old plantings containing much mild streak. The east side of the field was about 100 feet

[1] Scientific Contribution No. A177. Contribution No. 2078 of the University of Maryland Agricultural Experiment Station (Department of Botany).

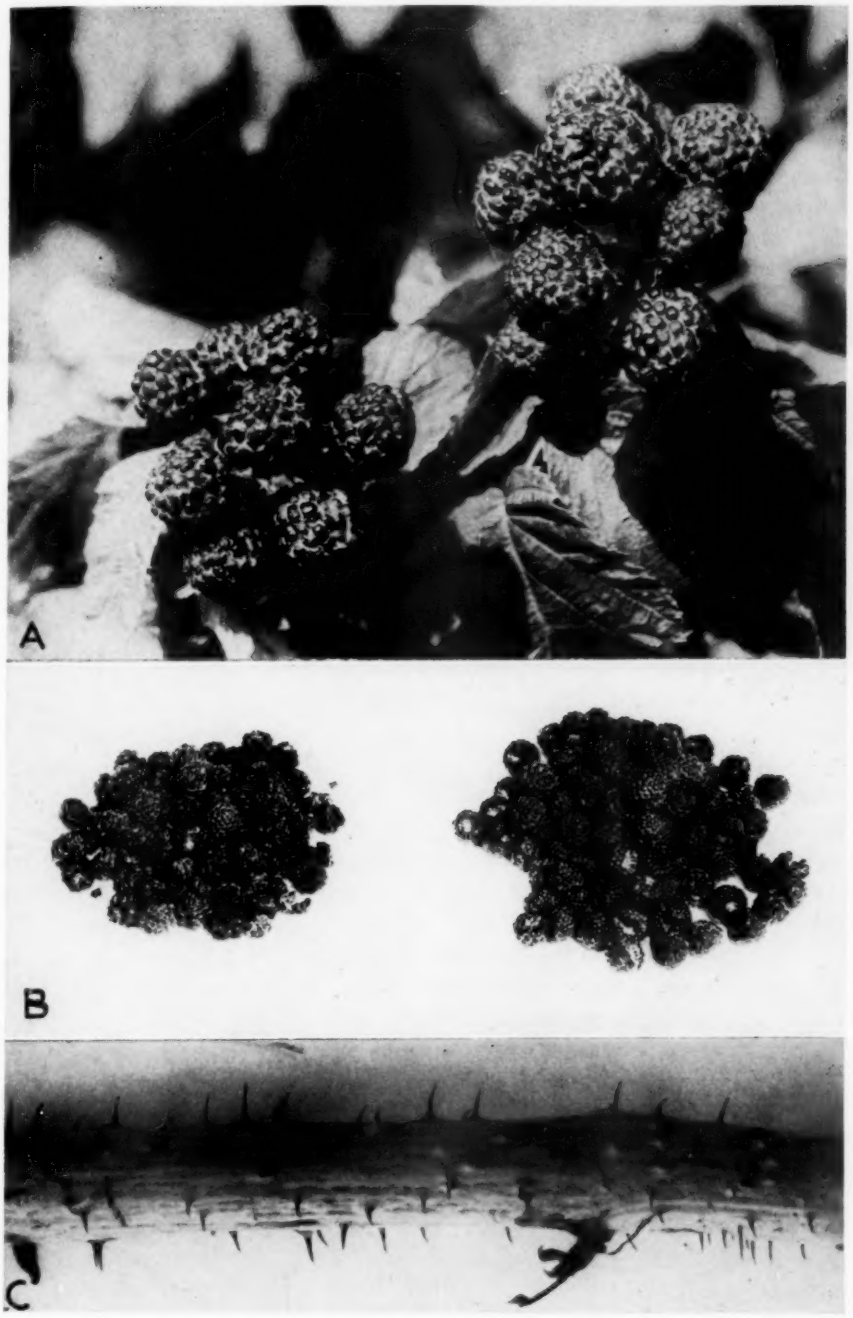

FIG. 1. Symptoms of mild streak disease on fruit (A and B) and cane (C) of the Cumberland black raspberry.

from a woods containing wild brambles. The land sloped rather steeply from the high southwest corner to the low northeast corner.

During the fruiting season of 1942 a careful inspection revealed that 37 of 2,435 plants were infected with mild streak. The infected plants were distributed rather uniformly throughout the field. These plants were burned, in place, with a flame thrower, and later removed. Thus it was apparent that some infection must have occurred during the growing season of 1941 or early in 1942 or that there was some mild streak present in the plants when set. Inspection during the rest of 1942 showed no evidence of

TABLE 1.—*Amount of mild streak in sections of test planting of Cumberland raspberries from 1942 through 1947*

Location	Plants observed	Year	Plants infected with mild streak	
	Number		*Number*	*Per cent*
Section 1	729	1942	12	1.6
Section 2	846		11	1.3
Section 3	860		14	1.6
Total	2435		37	1.5
Section 1	718	1943	417	58.1
Section 2	846		73	8.6
Section 3	860		57	6.6
Total	2424		547	22.6
Section 1	714	1944	481	67.4
Section 2	827		198	23.9
Section 3	852		132	15.5
Total	2393		811	33.9
Section 1	708	1945	586	82.8
Section 2	768		329	42.8
Section 3	832		217	26.1
Total	2308		1132	49.1
Section 1	709	1946	629	88.7
Section 2	704		449	63.8
Section 3	809		320	39.6
Total	2222		1398	62.9
Section 1	700	1947	670	95.7
Section 2	698		660	94.6
Section 3	804		681	84.7
Total	2202		2011	91.3

further infection. In 1943 the distribution of mild streak was again plotted. It was found that 547, or 22.6 per cent, of the 2,424 plants examined were infected. The increase in amount of infection was greatest in the north end of the planting. In view of the large amount of infection and because this planting was in cooperation with a commercial grower, roguing was discontinued.

Observations made as early as May 15, 1943, soon after new growth appeared, showed that the incidence of mild streak was much greater than in 1942. This early appearance of mild streak symptoms together with the fact that no further infected plants were recognized during the remainder of

the 1942 season suggests that current season infection cannot be readily recognized.

Spread of mild streak in different parts of the test planting was determined by dividing the fields into three sections. Section one included twenty rows at the north end; section two, the central twenty rows and section three, the twenty rows on the south end. The amount of mild streak found in each section for the six year period of 1942-1947 is shown in table 1.

The rapid spread of mild streak in section one has not been correlated with any factor but it seems possible that it may be related to the distribution of an insect vector and the proximity of wild and escaped brambles. Cooley (3, 4) and Rankin (6), on the basis of field observations, suggested that mild streak may be transmitted by an insect vector. Bennett (1) tested *Amphorophora rubi, Aphis rubiphila,* and *Amphorophora sensoriata* for their ability to transmit streak but obtained only negative results.

TABLE 2.—*Insects, belonging to groups known to transmit plant viruses, collected in test planting*

Aleyrodinae (2 species) Possibly Trialeurodes	*Erythroneura torella,* Rob.
Aulacaspis rosae (Bouché)	*Erythroneura sp.* Probably *obliqua* (Say)
Graphocephala coccinea (Forst.)	*Amphorophora sp.*
Scaphtopius acutus (Say)	*Cinara sp.*
Lepyronia quadrangularis (Say)	*Macrosiphum ambrosiae* (Thos.)
Empoasca erigeron, Del.	*Macrosiphum pisi* (Kalt.)
Empoasca fabae (Harris)	*Myzocallis punctatellus* (Fitch.)
Empoasca copula, Del.	*Cercosipha rubifolii* (Thos.)

Observations of insects present in this planting showed a stem-inhabiting aphid, tentatively identified as *Amphorophora sensoriata* Mason, to be the most prevalent aphid species present. Sweepings yielded a number of other insects. Those belonging to groups known to transmit plant viruses are listed in table 2.[2]

Of the various insects listed in table 2, only *Cercosipha rubifolii* (Thos.) is known to feed on *Rubus.* While the possibility that one of these or some other insect may transmit mild streak cannot be excluded it is felt that circumstantial evidence warrants further testing of *Amphorophora sensoriata.* Transmission experiments with this insect are now in progress.

SUMMARY

1. Mild streak, a disease apparently of virus nature, is widespread in Maryland black raspberry plantings.

2. Rapid spread of the disease may occur under field conditions.

3. Current season infection cannot be readily recognized.

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[2] The authors are indebted to the U. S. National Museum for identification of these insects.

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RESISTANCE TO BACTERIAL WILT AND BLACK SHANK IN FLUE-CURED TOBACCO[1]

T. E. SMITH AND E. E. CLAYTON

(Accepted for publication November 17, 1947)

INTRODUCTION

Bacterial wilt (*Bacterium solanacearum* E. F. Smith) and black shank (*Phytophthora parasitica* var. *nicotianae* Tucker) have caused serious diseases of tobacco for many years in restricted areas of the southeastern United States. Wilt occurred in North Carolina in the north central and eastern sections. Black shank occurred in the shade tobacco section of Florida and Georgia and in the flue-cured tobacco section of North Carolina and Virginia. Separate flue-cured tobacco varieties resistant to each disease have been developed and introduced. T.I. 448A from Colombia, South America, was shown to have high resistance to wilt (3) and in later work, Oxford 26, a resistant flue-cured variety, was selected from the cross of T.I. 448A × 400 (5). Florida 301 (6) was highly resistant to black shank in North Carolina and it was used in crosses with flue-cured varieties to develop Oxford 1, 2, 3, and 4 (2). These new varieties are grown successfully on soils infected with either bacterial wilt or black shank. However, in recent years black shank has spread into regions where wilt occurred. The situation now requires a variety highly resistant to both diseases. This paper is a report of resistance to black shank in genotypes resistant to bacterial wilt and of resistance to bacterial wilt in genotypes resistant to black shank. Results are also reported from a cross between wilt and black shank resistant genotypes.

RESULTS

Resistance to black shank was measured in replicated plantings made on infested field plots. To measure the degree of resistance to black shank, counts of dead or obviously diseased plants were made. Three wilt resistant genotypes (T.I. 448A, Oxford 26, and 43–2) were compared with Florida 301 which is black-shank resistant, and 4 flue-cured varieties (Gold Dollar, 400, 401, and Yellow Special) that are susceptible to both diseases. Approximately 3 months after transplanting, Florida 301 averaged 11 per cent black shank, and T.I. 448A averaged 49 per cent black shank. The difference between Florida 301 and T.I. 448A was significant (0.01). Oxford 26 and 43–2, F_6 lines of T.I. 448A × 400, averaged 86.5 per cent black shank and the four susceptible flue-cured varieties averaged 97.5 per cent black shank. The difference between the F_6 lines and the susceptible varieties was significant (0.01) in an analysis of variance of the transformed (1) data.

[1] Cooperative investigations of the Division of Tobacco, Medicinal and Special Crops, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, and The N. C. Agricultural Experiment Station and Department of Agriculture.

Thus all three wilt resistant genotypes tested had some resistance to black shank.

Resistance to bacterial wilt was also measured in replicated plantings on infested field plots. To measure wilt resistance, counts were made of plants dead or obviously wilted on 2 dates. Four black-shank-resistant genotypes (Oxford 1, 2, 3, and 201) were compared with wilt resistant Oxford 26 and two flue-cured varieties (Gold Dollar and 400) that were susceptible to both diseases. In midseason, 2 months after transplanting, all varieties had significantly (0.01) more wilt than Oxford 26. Among the black-shank-resistant genotypes, only Oxford 2 (51 per cent wilt) and Oxford 201 (54 per cent wilt) had significantly (0.01) less wilt than the average of the two susceptible flue-cured varieties (87 per cent). Near the end of the growing season, 4 months after transplanting, Oxford 2 was badly diseased. However, on this date, Oxford 201, an F_5 of Florida $301 \times 400 \times 400 \times 400$, averaged 63 per cent wilt in comparison to 15 per cent in Oxford 26 and 99 per cent in Gold Dollar. Thus, some wilt resistance occurred in 2 of the 4 genotypes resistant to black shank.

Lines resistant to wilt and to black shank were crossed in 1941. The hybrids were selected for wilt resistance and desirable growth characters in the F_2, F_3, and F_4. An F_5 wilt-resistant line, designated as Oxford 202, was grown in separate wilt- and black-shank-infested plots. On the wilt plots, 11 single plant selections of Oxford 202 averaged slightly less wilt than T.I. 448A under conditions where Gold Dollar was 100 per cent wilted. On the black-shank plots, these same lines of Oxford 202 averaged significantly (0.01) less black shank than Gold Dollar. In further tests of black-shank resistance in Oxford 202, nine F_6 lines averaged 47 per cent black shank in a planting where Florida 301 had 3 per cent and 400 had 100 per cent. These results on wilt-infested soil showed that very high resistance to wilt was recovered, and furthermore, many lines selected only for wilt resistance had moderate resistance to black shank.

DISCUSSION AND CONCLUSIONS

Resistance to bacterial wilt in T.I. 448A and resistance to black shank in Florida 301 are inherited on a multiple factor basis (2, 4, 6). Many field-plot data and farm experience have shown that the full resistance of each basic stock is necessary for effective disease control under conditions of continuous tobacco culture. The full resistance was difficult to recover after crossing. For example, in the F_2 of T.I. 448A $\times$ flue-cured varieties, 63 segregates of high wilt resistance were selected from a total population of 5200, a ratio of 1:82. In the F_2 of Florida 301 $\times$ flue-cured varieties, 29 segregates of high black-shank resistance were selected from a total population of 935, a ratio of 1:31. Based on these field-plot results, the F_2 of a cross between genotypes resistant to wilt and resistant to black shank should carry segregates of high resistance to both diseases in the ratio of 1:2655. In the absence of linkage, the development of a flue-cured variety highly resistant to both diseases would be an extremely difficult undertaking.

Linkage between wilt resistance and black-shank resistance was indicated by the results obtained since 1944. T.I. 448A, the source of resistance to wilt, was moderately resistant to black shank. After a cross of T.I. 448A $\times$ 400, selection for wilt resistance isolated F_6 lines with low resistance to black shank. Among black-shank-resistant varieties selected from backcross generations of Florida 301 $\times$ flue-cured varieties (recurrent parent), Oxford 2 and 201 had measurable resistance to wilt. Further evidence of linkage was obtained from the cross of a genotype resistant to wilt $\times$ a genotype resistant to black shank. The progeny of this cross was selected only for wilt resistance in the F_2, F_3, and F_4. This material, designated as Oxford 202, had high wilt resistance and moderate black-shank resistance in the F_5 and F_6. It is significant that the initial F_2, out of which Oxford 202 was selected, contained only 240 plants or approximately one-eleventh of the calculated number required for recovery of one F_2 segregate with high resistance to both diseases. Linkage increased the number of segregates with combined resistance. The results show that a flue-cured variety of tobacco with high resistance to wilt and black shank can probably be developed without resort to excessively large populations.

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CANNA MOSAIC IN THE UNITED STATES

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In September, 1942, Mr. Horace V. Wester of the National Capital Parks, Washington, D. C., submitted to the writers three samples of ornamental cannas (*Canna generalis* Bailey) as follows: Richard Wallace showing symptoms of a virus, the same variety apparently healthy, and the variety The President apparently healthy or symptomless. Two plants of *Canna glauca* L.[3] occurred in mixture with the mosaic Wallace, and showed strong yellow stripe symptoms. In December, 1945, Dr. George M. Reed supplied 5 varieties of canna previously selected at the Brooklyn Botanic Garden by Floyd F. Smith. These varieties were Indiana, King Humbert, Richard Wallace, Wyoming, and The President, all but the last showing symptoms of a virus disease. In nurseries in Michigan and Delaware visited by the writers in 1946 and 1947, all of 25 or more canna varieties grown except The President showed the same virus symptoms. We have attempted to determine whether this common mosaic of canna in the United States agrees with the mosaic of *Canna indica* L. previously described from the Philippines,[4] and whether The President is resistant or immune to this virus.

Occurrence of a mosaic of *Canna indica* in Japan was reported by Fukushi[5] in 1932, and by Ocfemia[6] in the Philippines in 1937. A detailed experimental study of the disease was made by Ocfemia, Macaspac, and Yuan,[4] who found it locally abundant in *C. indica* in the province of Davao. These workers describe and illustrate symptoms of canna mosaic in *C. indica* and in abaca (*Musa textilis* Nee). Early leaf symptoms are fine chlorotic lines connecting two branch veins. Later spindle-shape chlorotic areas may appear. In advanced stages continuous or broken chlorotic stripes extend from the midrib to the leaf margin parallel with the veins, and the leaf may become wrinkled or curled. Such stripes may become brown necrotic in late stages, particularly in ornamental cannas. Streaking of the petal color in *C. indica* and in ornamental varieties is also mentioned. Canna mosaic was not seed borne in *C. indica*. One successful transfer by leaf-rubbing was considered inconclusive. Transmission was successful

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[3] We are indebted to Dr. F. J. Hermann for identification of the species of *Canna*, *Hedychium*, and *Musa* mentioned in this paper.

[4] Ocfemia, G. O., I. S. Macaspac, and H. F. Yuan. Experimental transmission of the mosaic of *Canna indica*. Philipp. Agric. **30**: 357–370. 1941.

[5] Fukushi, T. A contribution to our knowledge of virus diseases of plants in Japan. Trans. Sapporo Nat. Hist. Soc. **12**: 130–141. 1932.

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with the aphids *Aphis gossypii* Glover and *A. maidis* Fitch, but not with *A. laburni* Kalt., *Pentalonia nigronervosa* Coq., or *Rhopalosiphum nymphaeae* L. Canna mosaic was transmitted to *C. edulis* Ker. as well as to *M. textilis*, *C. indica*, and ornamental cannas by *A. gossypii*, but this vector failed to transmit the virus to cotton or cucumber. *Aphis gossypii* was shown to transmit canna mosaic virus in the non-persistent manner, acquiring the virus in 5 minutes' feeding on a source plant and becoming non-infective after feeding on one healthy plant or after 1 hour without food.

MATERIALS AND METHODS

The canna varieties listed above served as sources of the virus. The procedures used in mechanical transmission and in insect transfer experiments were the usual ones that we described earlier.[7] Canna seedlings were used as test plants. *Canna glauca* which seemed promising for this purpose, failed to set seed in our cultures even when hand-pollinated. Seed of Richard Wallace open pollinated in the field produced virus-free seedlings, but these proved somewhat inferior for test purposes because of weak symptom expression when infected. In December, 1945, we received seed of *C. indica* from Trinidad, through the courtesy of Mr. B. Y. Morrison of the Division of Plant Exploration and Introduction. These seeds germinated readily after scarifying and produced satisfactory test plants. Test plants of *Hedychium coronarium* Koenig were divisions of individuals purchased from a nursery in Alabama, and divisions of *Musa cavendishii* Lamb. and *M. textilis* were obtained from stock growing in the greenhouses at Plant Industry Station, Beltsville, Maryland.

IDENTITY OF THE VIRUS

There is complete agreement in the symptoms observed in our material of *Canna indica* (Fig. 1, A) and ornamental cannas (Fig. 1, B) with those described by Oefemia, Macaspac, and Yuan for these species in the Philippines. Similar symptoms occurred in *C. glauca* (Fig. 1, C) with necrotic streaks prominent in the expanded leaves. This species was not included in the Philippine study and their *C. edulis* was not available to us. Repeated inoculation of *Hedychium coronarium*, *Musa textilis*, and *M. cavendishii* by suitable aphid vectors has failed to induce symptoms in these species in our trials, and *Myzus persicae* (Sulz.) also failed to recover the virus from inoculated *M. textilis*. As previously noted, Oefemia, Macaspac, and Yuan readily infected *M. textilis* in the Philippines. This discrepancy is possibly not significant, as it is doubtful whether our potted *M. textilis* ever attained the vigorous growth that this species makes in the open in the tropics. Canna mosaic was not transmitted to cucumber in two trials, nor *Vicia faba* L. in one trial. In comparative tests *M. persicae* transmitted cucumber mosaic to cucumber and to *C. indica*, but transferred canna

[7] Smith, Floyd F., and Philip Brierley. Ornithogalum mosaic. Phytopath. **34**: 497–503. 1944.

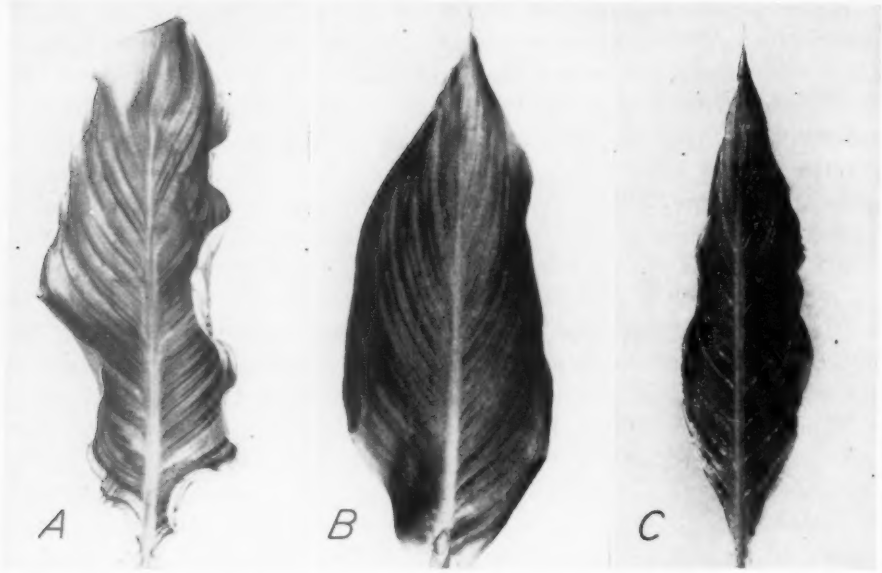

FIG. 1. Symptoms of canna mosaic. A. *Canna indica;* B. Ornamental canna variety Richard Wallace; C. *Canna glauca.* Photos by O. H. Greeson.

mosaic to *C. indica* only. Cucumber mosaic was reisolated mechanically in tobacco from cucumber and from *C. indica*, but no transfer of canna mosaic from *C. indica* resulted in a parallel test on tobacco. Initial symptoms of canna mosaic in *C. indica* have been noted 4 times after intervals

TABLE 1.—*Transmission of canna mosaic by aphids*

No. of trials	Source of virus	Plants inoculated	Plants affected
Aphis gossypii			
2	*Canna glauca*	*C. generalis*	0/15
1	*Canna glauca*	*C. indica*	0/3
1	Richard Wallace	*C. indica*	1/5
Aphis maidis			
2	*C. glauca*	*C. generalis*	7/15
Macrosiphum solanifolii			
1	Richard Wallace	*C. indica*	2/5
1	*C. glauca*	*C. indica*	0/5
Myzus circumflexus			
1	*C. glauca*	*C. indica*	1/5
Myzus convolvuli			
1	Richard Wallace	*C. indica*	0/5
Myzus persicae			
2	*C. glauca*	*C. generalis*	4/13
1	*C. glauca*	*C. indica*	0/2
3	*C. indica*	*C. indica*	10/12
1	King Humbert	*C. indica*	4/5
2	The President	*C. indica*	0/8
4	Richard Wallace	*C. indica*	15/20
1	Richard Wallace	The President	0/2
2	Wyoming	*C. indica*	5/9

of 13 to 15 days, but in many plants symptoms were first detected after 20 to 40 days. This is good agreement with the Philippine report of 2 weeks stated by Ocfemia, Macaspac, and Yuan.

No evidence of seed transmission was detected when 44 seedling *Canna indica* were grown from seed of mosaic plants; Ocfemia, Macaspac, and Yuan also report no seed transmission. Mechanical transmission by the carborundum leaf-rubbing method failed on 10 *C. indica* plants in one trial, but produced typical canna mosaic symptoms in 4 of 25 plants inoculated in later trial from *C. indica* to more succulent test material of *C. indica*. Ocfemia, Macaspac, and Yuan reported one mechanical transfer to *Musa textilis*, which they considered of doubtful significance.

Aphid transmission tests in cannas are summarized in table 1. All tests were conducted with the technique suited to nonpersistent viruses, the successful transfers, therefore, confirming the nonpersistent nature of canna mosaic virus which the Philippine workers had previously reported. Transmission by *Aphis gossypii* and by *A. maidis*, previously shown to be vectors, was successful. However, *A. gossypii* was comparatively inefficient as a vector in our trials, although very efficient in the Philippines. Three additional vectors, *Macrosiphum solanifolii* (Ashm.), *Myzus circumflexus* (Buckt.), and *M. persicae* were established, but *M. convolvuli* (Kltb.) failed to transmit canna mosaic in a single trial. *Myzus persicae* proved to be the most efficient of the species used in our trials, and was, therefore, used in most of our attempts to establish additional hosts outside the genus *Canna*, all of which were unsuccessful.

IMMUNITY OF THE PRESIDENT CANNA

The variety The President was symptomless as received from Washington, D. C., and from Brooklyn, N. Y., in each of which places it had been grown adjacent to mosaic cannas. No mosaic symptoms have been detected in this variety in the writers' scattered observations elsewhere. In parallel tests *Myzus persicae* transmitted canna mosaic to *Canna indica* from the varieties King Humbert (4/5), Richard Wallace (7/10), and Wyoming (5/5), but failed to transmit from The President (0/5). The President was then inoculated from Richard Wallace by this vector without effect (0/2) while *C. indica* in a parallel trial was infected (3/3). Transfers of *M. persicae* from these inoculated plants of The President to *C. indica* were without effect (0/8), but parallel transfers from mosaic *C. indica* to *C. indica* produced typical canna mosaic symptoms (3/3). These experimental results show that The President is not a symptomless carrier of canna mosiac, and offer strong evidence that this variety is immune from the disease.

DISCUSSION AND CONCLUSIONS

Canna mosaic as studied by the writers is considered the same as the mosaic of *Canna indica* previously reported from the Philippines. Transmission by *Aphis gossypii* and by *A. maidis* was confirmed. The principal

discrepancy is the failure of our virus to infect *Musa textilis*. *Canna glauca* is reported as an additional host species. Experimental transmissions by leaf rubbing and by additional vector species *Macrosiphum solanifolii*, *Myzus circumflexus*, and *M. persicae* were established. Evidence is presented that the popular red-flowered ornamental canna variety The President is immune from canna mosaic.

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INFECTION OF TOMATO FRUITS BY COLLETOTRICHUM PHOMOIDES[1]

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(Accepted for publication November 21, 1947)

The fungus *Colletotrichum phomoides* (Sacc.) Chester has long been considered a wound pathogen on ripe tomatoes. Arthur (1), Chester (2, 3, 4), Halsted (9), Gueguen (8), and others were able to transmit the disease from one ripe tomato to another only by wounding the inoculated fruits. Davidson (5) concluded from field observations that natural infection must take place through microscopic cracks in the cuticle of the fruits. Doolittle (6, p. 27) stated that *C. phomoides* can infect apparently unwounded tomatoes. This statement was based on studies in which healthy tomatoes were sprayed with a suspension of spores of the anthracnose fungus.[3]

Tomato anthracnose develops ordinarily on ripe fruits but the failure to control the disease by application of fungicides to near-ripe or ripe fruits suggests that infection takes place some time prior to ripening (16, 17).

A study was made of some of the factors related to infection of tomato fruits by *Colletotrichum phomoides*, with emphasis on the mode of infection and the stages of maturity at which the fruits are susceptible to infection.

METHODS

Experiments were conducted on greenhouse-grown tomatoes using two red-fruited varieties, Early Baltimore and Garden State. In preliminary studies field-grown tomatoes proved unsatisfactory because of the high percentage of natural infection. The greenhouse plants were started in pots and after several weeks were transplanted to the bench, where they were supported by heavy twine and pruned to two branches. A date tag was attached to each blossom shortly after pollination when the ovaries became slightly swollen.

The inoculum used was obtained from five-day-old cultures of *Colletotrichum phomoides* on potato-dextrose agar slants. A small amount of water was added to each slant and a spore suspension prepared by gently scraping the surface with a wire loop. Spores were then washed[4] by cen-

[1] Condensed from a thesis submitted in partial fulfillment of the requirements for a degree of Doctor of Philosophy in the Graduate School of the University of Illinois.

[2] The writer is indebted to Dr. M. B. Linn for advice during the progress of the work and criticism of the manuscript. Grateful acknowledgement for criticism of the manuscript is also made to Professor N. E. Stevens.

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[3] Correspondence with Dr. Doolittle.

[4] Washing of the spore suspension was essential especially when green tomatoes were inoculated. Unwashed spore suspensions or agar discs on which the fungus was growing contained materials which diffused from the inoculum into the green fruits causing black spotting and, later, distortion of the fruits.

trifuging through two changes of water. Several ml. of this suspension were poured onto pads of moist cotton approximately two inches in diameter and these were applied to the fruit surfaces. Detached fruits were placed in moist chambers for two days. Fruits inoculated on the vine were covered with waxed paper bags for two days to conserve moisture.

In histological studies of the mode of infection, fruits were inoculated by the methods just described or in some cases with spore suspensions applied in droplets to marked areas on the fruits. Inoculated areas were cut from the fruits at various time-intervals ranging from twenty-four hours to several weeks. The specimens were fixed in FAA, dehydrated in ethyl alcohol, cleared in xylol, and imbedded in paraffin according to the usual methods. Sections were cut 10 microns in thickness and stained with safranin and fast green. A few sections were stained with Flemming's triple stain after decolorizing in Stockwell's solution. Temporary mounts for observation of the spores and appressoria on the fruit surfaces were made by mounting pieces of tomato skin in lacto-phenol containing 0.1 per cent cotton blue.

FRUIT INFECTION

Wounding

In studying the relation of wounding to infection five shallow wounds were made with a sharp needle in the epidermis of each tomato at the time of inoculation. Fruits of various ages were inoculated and allowed to ripen fully on the vines. They were then picked and stored at 80° F. for two weeks. No attempt was made to control humidity. As the lesions appeared care was taken to determine whether the lesions developed from wounds or in the surrounding apparently unwounded areas. The percentages of fruits developing lesions from wounds and the percentage showing lesions in the unwounded areas are presented in table 1.

TABLE 1.—*Infection of wounded tomato fruits*

Variety	Age when inoculated	Number inoculated	Percentage developing lesions from wounds	Percentage developing lesions in unwounded areas
Early Baltimore	10 to 20 days	8	0	50
	20 to 40 days	5	60	80
	40 to 60 days	11	100	72
	Control, uninoculated	16	.....	0
Garden State	10 to 20 days	10	0	70
	20 to 40 days	8	25	87
	40 to 60 days	8	62	100
	Control, uninoculated	15	.....	0

These data indicate that as the fruits approach maturity the pathogen can readily gain entrance through wounds. Young fruits, however, are not easily infected in this manner. Wounding of young fruits causes a corking of the surrounding tissues which effectively prevents further development of the fungus. Wounding, in the manner described, is not necessary to obtain infection. Inoculation of young fruits produces typical lesions only on apparently unwounded areas. Infection of mature fruits can also occur on unwounded areas.

Maturity

The relation of maturity of fruits to the susceptibility to infection by

TABLE 2.—*Infection of unwounded tomato fruits by Colletotrichum phomoides in relation to the age of the fruits when inoculated*

Variety	Age when inoculated	Number inoculated	Percentage of green fruits with stylar rot	Percentage of ripe fruits with typical lesions
Early Baltimore	5 days	8	25	0
	10 days	10	20	0
	15 days	13	7	33[a]
	20 days	14	0	57
	30 days	17	0	88
	40 days	25	0	80
	50 days plus, fruits green	21	0	85
	50 days plus, fruits pink to ripe	15	0	80
	Control, uninoculated	16	0	0
Garden State	5 days	4	75	0
	10 days	13	30	44[a]
	15 days	16	0	62
	20 days	23	0	65
	30 days	11	0	63
	40 days	14	0	85
	50 days plus, fruits green	14	0	92
	50 days plus, fruits pink to ripe	7	0	85
	Control, uninoculated	15	0	0

[a] Calculated on the basis of the fruits which reached maturity.

Colletotrichum phomoides was investigated by inoculating unwounded tomatoes at various ages, viz., 5, 10, 15, 20, 30, 40, 50 days, and later as the fruits colored and ripened. The fruits were picked and stored for two weeks in the same manner as previously described.

The percentages of infection (Table 2) indicate that the fungus can penetrate the cuticle and cause incipient infection at a very early stage in fruit

development. There is some increase in susceptibility to this type of infection as the fruits mature.

A certain number of the fruits inoculated when 15 days old or less developed a stylar rot 20 to 30 days after inoculation. The area around the style became black to dark brown and black sclerotial bodies of the fungus developed in this dry, rotted region. The rot was much more extensive in the interior of the fruits than it was on the exterior. Seeds, as well as the tissues of the walls of the seed cavities, showed discoloration and rotting. *Colletotrichum phomoides* was repeatedly isolated from the affected tissues. Rotting in this instance may have followed injury to the styles at the time of inoculation.

Temperature Relation

Nightingale and Ramsey (13) reported that *Colletotrichum phomoides* was unable to grow on culture media or in wounded tomatoes at temperatures above 95° F. The optimum temperature for growth of the fungus, computed from 6 days' development, was shown to be 80° F. The present work shows that infection of apparently uninjured fruits is obtained over a similar wide range.

Unwounded, ripe tomatoes were inoculated by the method previously described and placed in moist chambers for two days. During inoculation and subsequent storage, fruits were kept at 50, 60, 70, 80, 90, and 100° F. At 50° F. lesions developed slowly after approximately twenty days. Infection was readily obtained at 60 and 70° F., lesions being produced in five to eight days. Most rapid development of lesions was at 80° F. At this temperature they often appeared within three days. Lesions produced at 80° F. and below were similar in appearance to those on field-infected fruits. A few lesions developed at 90° F. after approximately seven days. These few lesions were very small, sunken, and dry. No lesions developed at 100° F.

The time required for the appearance of lesions was not constant for any one temperature. The rapidity with which lesions developed was dependent not only upon temperature but also upon maturity of the fruits at inoculation. Large green tomatoes could be infected in the manner just described but the appearance of the lesions in this case was delayed until after the fruits had ripened.

HISTOLOGICAL STUDIES ON PENETRATION AND INFECTION

Fungi of the genera *Colletotrichum* and *Gloeosporium* characteristically enter the host plant by means of penetration tubes which develop below appressoria. Simmonds (15) studied in detail the penetration of fungi causing the anthracnose diseases of banana, papaw, and mango. In these cases an extremely fine thread develops from a short peg-like projection at the base of the appressorium. The fine thread penetrates the cutin layer and broadens in contact with the cellulose wall of an epidermal cell to form a well-defined hyphal mass between the cutin layer and the cell wall.

Spores of *Colletotrichum phomoides* germinate readily in droplets of water either on tomato fruits or on glass slides. At varying intervals after germination the fungus forms appressoria on the hyphae. On tomato fruits the appressoria may form on the ends of very short hyphae protruding from germinating spores, or they may develop after extensive mycelial growth. Commonly more than one appressorium is formed from a single spore.

Studies of stained sections prepared from inoculated tomatoes show that a penetration tube grows from the lower side of the appressorium into the cuticle (Fig. 1, A, Fig. 2, C, D). This tube is constricted at the point of entry into the cuticle and is swollen midway through the cutin layer. A nucleus is often evident in the penetration tube soon after the tube grows into the cutin layer (Fig. 2, D). Upon contact with the host cell wall the tip of the penetration tube enlarges slightly, pushing the host cell wall inward and away from the cutin layer. These steps in the penetration of the cutin layer have been observed in tomato fruits varying in age from fifteen days to red-ripe maturity.

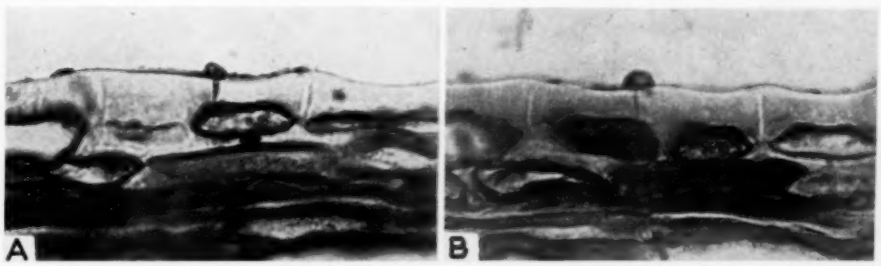

FIG. 1. Penetration beneath appressoria of the cuticle of a Garden State tomato 60 days old, 6 days after inoculation. A. Penetration tube has grown through the cutin layer and is in contact with the epidermal cell wall. B. Fungus hyphae developing from a penetration tube. The hyphae have pushed aside the cell wall and are growing between the cell wall and the cutin layer. The cell contents and the walls and contents of adjacent cells are strongly stained with safranin. Photographs approx. 408 ×.

In green fruits the growth of the pathogen is limited to the area between the cutin layer and cell wall. The cell wall in contact with the penetration tube is heavily stained by safranin. The contents of the affected cell often take the stain more strongly than the adjacent cells. A small swelling in the cell wall usually develops on the side opposite the fungus.

In ripe fruits the hyphae, which have penetrated the cuticle either recently or at some earlier stage when the fruits were green, continue enlargement between the cutin layer and the host cell wall (Fig. 2, E). Lateral development from one epidermal cell to another is apparently prevented by the cutin deposited between the cells. The wall in contact with the fungus is pushed aside by several swollen hyphae. The fungus slowly dissolves the cell wall and the contents are pushed into a small mass at the base which stains with safranin (Fig. 1, B). Adjacent cell walls in the hypodermis and epidermis also show this characteristic staining in advance of the fungus.

From the epidermal cell the fungus grows into the hypodermal layers but never laterally into the adjacent epidermal cells. The development is both inter- and intracellular. When it is intracellular the contents of the cell are pushed aside. The hyphae are swollen, with some constrictions, and limited in extent. At this stage there is a certain amount of lateral development in the hypodermis. The fungus continues to cause discoloration of cell walls and contents in advance of its development.

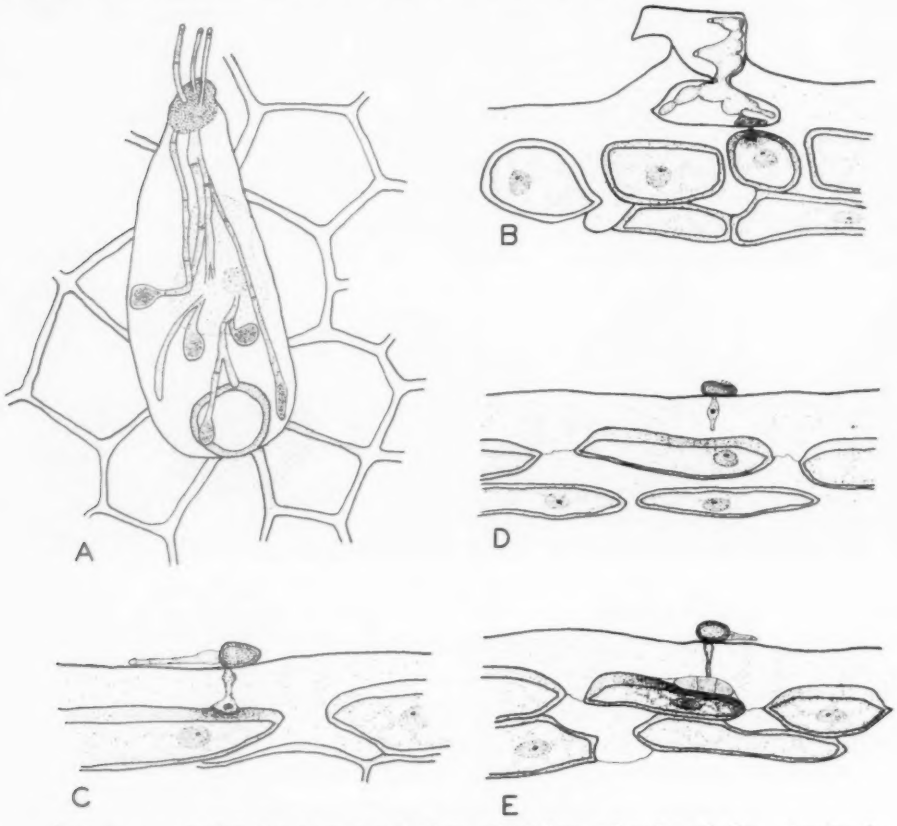

FIG. 2. A. Garden State tomato 28 days old, 2 days after inoculation, showing invasion of broken epidermal hair by hyphae. B. Early Baltimore tomato 33 days old, 4 days after inoculation, showing development of hyphae in epidermal hair and penetration beneath appressorium at base of hair. C. Same tomato showing penetration tube growing through cutin and swelling in contact with cell wall. D. Garden State tomato 60 days old, 6 days after inoculation, showing penetration tube in cutin. E. Same tomato showing swollen hyphae developing between cutin and cell wall. Drawn with aid of camera lucida, approx. 573 ×.

The hyphae change in form and manner of growth when they enter the parenchyma cells below the hypodermal layer. Growth is rapid and more extensive than that observed in the epidermis and hypodermis. The hyphae ramify in all directions showing both intra- and intercellular development. The cell walls and cell contents are penetrated readily and the fungus no longer follows the contours of the cell walls. The hyphae are much finer

than those in the hypodermal layer and are no longer constricted. The cell walls and cell contents are not discolored in response to the fungus. At this stage there is a partial collapse of the cells of the hypodermal layer and the affected parenchyma cells. As the lesion enlarges the fungus continues its development in the parenchyma cells and may also develop laterally in the hypodermal layer and in the cells of the epidermis.

It was noted in temporary mounts of the skin of inoculated fruits that the fungal hyphae were often associated with the epidermal hairs. The fungus penetrated and developed within the cells of some hairs (Fig. 2, A). The hyphae were generally tipped with appressoria near the base of the hair. On ripe fruits lesions occasionally appear to develop from the base of such affected hairs. This penetration and development seems to be essentially the same as that described for cuticular penetration. The fungus forms appressoria at the base of the hair from which it penetrates to adjacent cells of the hypodermal layer (Fig. 2, B). In green fruits the growth of the fungus is arrested at the first cell it contacts beneath the hair. It should be emphasized that the common method of entry of the pathogen is directly through the cuticle, although the epidermal hairs on occasion appear to be infection courts.

Various types of cracks in the cuticle of field-grown tomato fruits have been reported by Young (18) and Groth (7). Davidson (5) has suggested that these are the primary infection courts for *Colletotrichum phomoides*. In the course of the present studies cracks were rarely observed in the cuticle of greenhouse-grown tomatoes. Since the fungus appears to be admirably suited for direct penetration of the cuticle, it is unlikely that cuticular cracks are important in penetration and infection of the fruits.

RELATION OF pH CHANGES IN FRUIT TO GROWTH OF PATHOGEN

One of the striking features of tomato anthracnose is that lesions develop only on ripe or ripening fruits. Since the fungus can exist within or beneath the cutin layer from the time the fruit is 10 days old until maturity, it must be assumed either that there are factors present in green tomatoes which inhibit further development of the fungus or that some material necessary for fungal growth develops during the ripening process. It has been suggested that changes in pH may be a controlling factor. There is no evidence that this is the case.

Nightingale and Ramsey (13), working with tomato anthracnose, concluded that other changes in the fruit during ripening are more important than changes in acidity in influencing the development of decay. They found that the average pH of the green tomatoes which they studied was 4.7 and that of ripe tomatoes 6.01. Their conclusions were based on the growth of *Colletotrichum phomoides* on potato-dextrose agar adjusted to these two values.

Hamner and Maynard (10), reviewing the factors influencing the nutritive value of the tomato, reported that figures of various workers on the pH

of ripe tomatoes range from 3.8 to 4.61. Hoffman, Krause, and Washburn (11) stated that acidity increases steadily during the period of fruit enlargement, and most rapidly as the fruits approach full size, but decreases during the ripening process. They did not report the pH of the fruits at various stages of maturity.

In the present investigation, tomatoes of various ages were macerated individually in a Waring Blendor and portions of this material were used to determine the pH with a Beckman pH Meter. In some tests certain sections of individual fruits were blended with a mortar and pestle and the pH determined in a similar manner.

Tomatoes less than 25 days old have a pH between 4.7 and 5.05. During the subsequent development there is a slow decrease in pH which lasts 20 to 30 days depending on the variety. This is followed by a sharp decrease to a point between pH 3.9 and 4.2 just prior to the development of red color

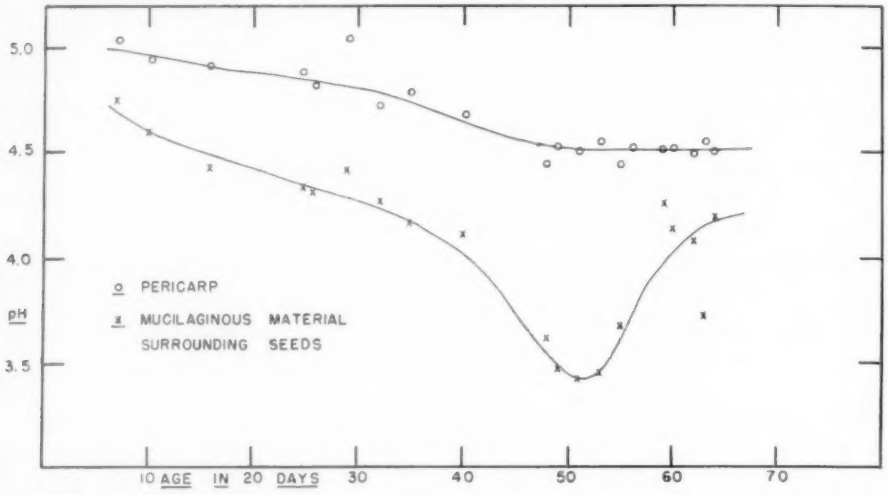

FIG. 3. The pH of the pericarp and the mucilaginous material surrounding seeds of Early Baltimore tomatoes of various ages.

in the fruits. The pH rises as the red color deepens so that mature tomatoes are between pH 4.2 and 4.6. There seemed to be slight consistent variations between the two varieties used but in the present work these differences were considered too minor to enter into the problem and could not be correlated with any differences in the amount of anthracnose.

Since the histological investigations demonstrated that the anthracnose fungus is confined mainly to the outer layers of the green fruits, the pH of various sections of Early Baltimore fruits was determined. The pericarp of the green and ripe fruits was found to have a consistently higher pH than the mucilaginous material around the seeds (Fig. 3). The pH of the pericarp drops slowly during the development and maturation of the fruit. On the other hand, the mucilaginous material surrounding the seeds shows a marked decrease in pH as the green fruits approach full size. During the

change from green to red-ripe the pH of this material rises and approaches that of the pericarp. This would indicate that the changes in pH noted in the whole fruits during maturation are the result of the changes in the interior of the fruits and not in the outer layers. It is in these outer layers that the anthracnose fungus remains apparently latent following penetration of the cutin layer. The inner walls of the carpels and the placental tissue have a pH between the two values presented in figure 3.

A series of cultural studies was run to determine the growth of *Colletotrichum phomoides* on media with a wide pH range. The fungus was grown on potato-dextrose agar containing 2.5 gm. of K_2HPO_4 per liter. The pH was adjusted to various levels by aseptic addition of 50 per cent citric acid

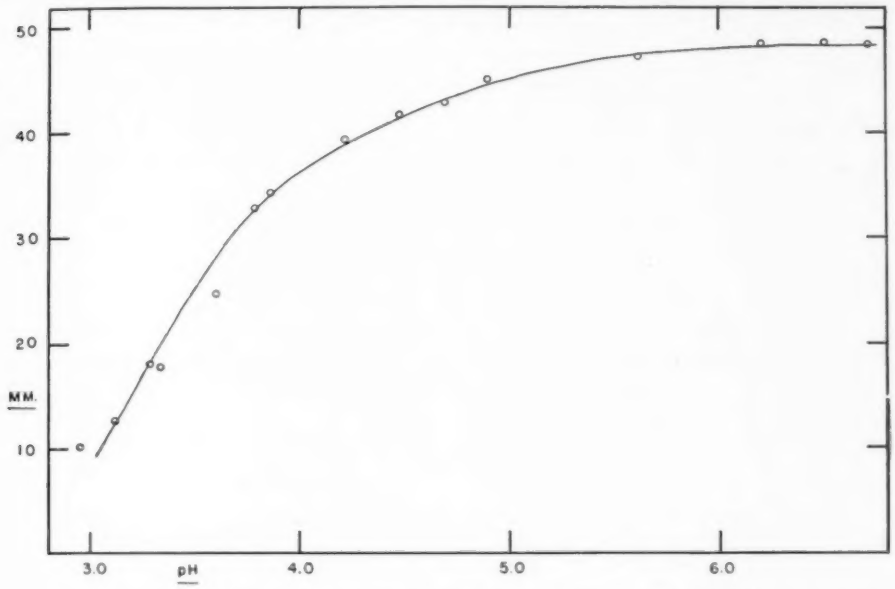

FIG. 4. The relation of pH to the growth of *Colletotrichum phomoides* on potato-dextrose agar. Diameter of colonies after five days at 80° F.

just before the agar was poured into Petri dishes. While the plates were being poured the pH of each medium was determined with a Beckman pH Meter. The agar was inoculated with a disc 1 mm. in diameter from the edge of a young colony of *C. phomoides*. The cultures were incubated at 80° F. for five days. The size of the colonies was determined by averaging the extreme diameters.

The pathogen grows well in culture within the extremes of pH found in tomato fruits (Fig. 4) and is not markedly inhibited unless the pH is lower than that found in green or ripe fruits. The range of pH found in the pericarp of tomatoes, pH 4.5 to 5.0, appears to be admirably suited to the growth of *Colletotrichum phomoides*.

It is apparent that the changes in pH which occur in the fruits during ripening do not influence directly the development of anthracnose on ripe

tomatoes. The inoculation studies demonstrated that *Colletotrichum phomoides* may occasionally penetrate at the style of the very young fruits and develop in the interior. In this stylar infection, the pericarp, and in particular the hypodermis and epidermis, are the last tissues to be invaded by the fungus. The inner tissues of the green tomatoes have a pH at which minimum development of the fungus would be expected. For this reason there must be factors in the pericarp other than pH which inhibit the growth of the fungus in green fruits.

Since the anthracnose fungus is confined to the epidermis and hypodermis during the latent stages of infection and no attempt was made to separate these tissues from the remainder of the pericarp, it is obvious that the pH of the immediate environment of the fungus has not been determined.

DISCUSSION

The present studies show that the pathogen causing tomato anthracnose is capable of penetrating the cuticle of green as well as ripe tomatoes, although lesions are produced only on ripe fruits. In green fruits the fungus remains latent until ripening begins. The fungus is able to push between the cell wall and the cutin layer to a limited extent but there is no evidence that it is capable of dissolving or penetrating the wall while the fruit is still green. The growth of the fungus in green fruits may be restricted by the composition of the cell walls. These cell walls, in addition to cellulose, are composed of protopectin which is partially converted to pectin in the ripening process. Simmonds (15) suggested that the latent condition of the banana anthracnose fungus in green fruits may be caused by the inability of the fungus to attack the protopectin of the cell walls. During ripening the protopectin is converted to soluble pectin which the fungus is presumably able to attack. Le Crone and Haber (12) have shown that protopectin is converted to soluble pectin during ripening of tomato fruits.

Sando (14) discussed the process of ripening in the tomato and pointed out that the most striking change which occurs is that undergone by the carbohydrates. Sugars increase during the maturation of the fruit and starches decrease during the same period of time. These changes, however, can not be expected to affect drastically a fungus which exhibits very little selectivity in regard to the source of carbon in its nutrition.

The relative amount of moisture is probably the controlling factor influencing infection under field conditions since the fungus can infect the fruits over a wide range of temperatures. The fungus can continue development in the ripe fruits over a similar wide temperature range. Therefore, if the fruits are infected in the field, little can be done by controlling storage temperatures to prevent the development of lesions as the fruits mature. Treatment of the harvested fruits with fungicides prior to storage can be expected to give little appreciable control of anthracnose since the fungus is latent beneath the cutin layer.

Wounds occurring naturally or produced in handling can not be considered an important factor in the development of this disease. Cultural practices which reduce wounding or breeding work to minimize cuticular cracking can not be expected to produce reductions in the amount of anthracnose. The pathogen penetrates the uninjured cuticle with ease and it is likely that this type of infection is responsible for serious outbreaks of the disease.

Since the fungus is capable of remaining latent in green tomato fruits, effective control with fungicides is dependent upon good coverage of each fruit throughout the entire period of its development on the vine. Sprays and dusts applied at the time the fruits are ripening may give a slight reduction in the amount of anthracnose but such treatment can not be expected to affect the latent infection which may already be present in the fruits.

SUMMARY

A study was made of some of the factors related to infection of tomatoes by the anthracnose fungus, *Colletotrichum phomoides*, using greenhouse-grown fruits.

When the pathogen was introduced through wounds in fruits on the vine, infection was readily obtained on large green or mature fruits. Infection was obtained on unwounded areas of tomato fruits less than 20 days old but not through wounds on these fruits.

Unwounded tomatoes were susceptible to infection from the time the fruits were 10 days old until maturity. The susceptibility to infection increased with the age of the fruits. The fungus, in green fruits, remained latent until the fruits ripened and produced typical lesions at that time.

Detached, mature, unwounded tomato fruits were readily infected through a wide range of temperatures below 90° F. Lesions were produced most rapidly at 80° F.

Colletotrichum phomoides penetrates the cuticle of the fruit by means of a penetration tube which grows from the lower side of the appressorium. The infection tube in green fruits swells slightly on contact with the cell wall but remains latent between the cell wall and the cutin layer. As the fruits ripen the fungus enlarges and produces swollen, inter- and intracellular hyphae in the hypodermis. Upon contact with the parenchyma cells the fungus develops many fine hyphae which grow rapidly through the walls and cell contents. Microscopic cracks in the cuticle are not believed to be a factor in infection.

There is no apparent relation between the pH of the fruits during ripening and the development of lesions. The fungus grows well within the ranges of pH found in tomatoes.

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ANTHRACNOSE OF TOMATO[1]

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INTRODUCTION

The anthracnose fungus was described on tomato (*Lycopersicon esculentum* Mill.) by Saccardo (13) in 1878 as *Gleosporium phomoides*. It was first noted in the United States in 1891 by Chester (2), who, believing the fungus to be distinct from that of Saccardo, since setae were produced in the acervulus, designated it as *Colletotrichum lycopersici*. Further investigation, however, revealed that the organism varied widely with regard to production of setae, sometimes none being produced. After two years of study Chester concluded that the American organism was the same as that described by Saccardo and in accord with rules of nomenclature he named it *Colletotrichum phomoides* (Sacc.) Chester (3). Gueguen (6) observed the disease in France in 1892.

Although anthracnose has been of frequent occurrence on the tomato, particularly in the canning region from New Jersey to Missouri, little research has been reported on the life history of the causal organism or upon the disease cycle. The purpose of this investigation was to study the method of overwintering of the fungus, the source of primary inoculum, the disease cycle on foliage and fruits, the host range of the fungus and the influence of various factors upon the development of the organism in pure culture.

THE CAUSAL ORGANISM

Source and Variability of Cultures

Isolates from 147 naturally infected tomato fruits from Racine and Kenosha, Wisconsin, showed a considerable range of growth characters on potato-dextrose agar.[2] All fell within the characteristics of acervulus and spores described by Chester (3). Some 14 fairly distinct types of cultural characteristics were recognized. In most of these, spores were not produced abundantly enough to accumulate in conspicuous spore masses but all produced some spores. They appeared to be chiefly of the MC type described by Tompkins and Hansen (15) for *Gleosporium thumenii* (Sacc.) var. *tulipae* Tompkins and Hansen. Isolates also varied in the color of the substrate; in prostrate or aerial growth of mycelium; and in the number, size, and arrangement of the stromata. In general the variability appeared to be of the same kind as that reported for *Colletotrichum destructivum* O'Gara by Chilton (4), for *C. capsici* Syd. by Ramakrishnan (11), and for *G. thumenii* var. *tulipae* by Tompkins and Hansen (15).

Three isolates were selected for use in this study. Their growth on potato-dextrose agar is characterized as follows:

[1] The writers are indebted to Eugene Herrling for preparing the illustrations, and to John H. Owen for assistance in some of the field work.

[2] 200 gm. potato; 20 gm. dextrose; 17 gm. agar; 1000 ml. distilled water.

Cp1 (Fig. 1), isolated from tomato, produced aerial mycelium and relatively large stromata scattered uniformly over the surface of the agar. Spores were produced sparsely and were similar in size and shape to those of Cp5. It was similar to the MC type of Tompkins and Hansen (15).

Cp4 (Fig. 1) was isolated from naturally infected tomato by W. J. Hooker. This isolate was distinct from all other strains in that the stromata were white in contrast with the black stromata of other isolates. The white mycelium was submerged; sporulation was profuse and pink masses of spores were conspicuous on the surface of the culture. The spores were straight with subacute ends, continuous, and uninucleate and they each contained two or three vacuoles. Measurements of 100 spores from each of five

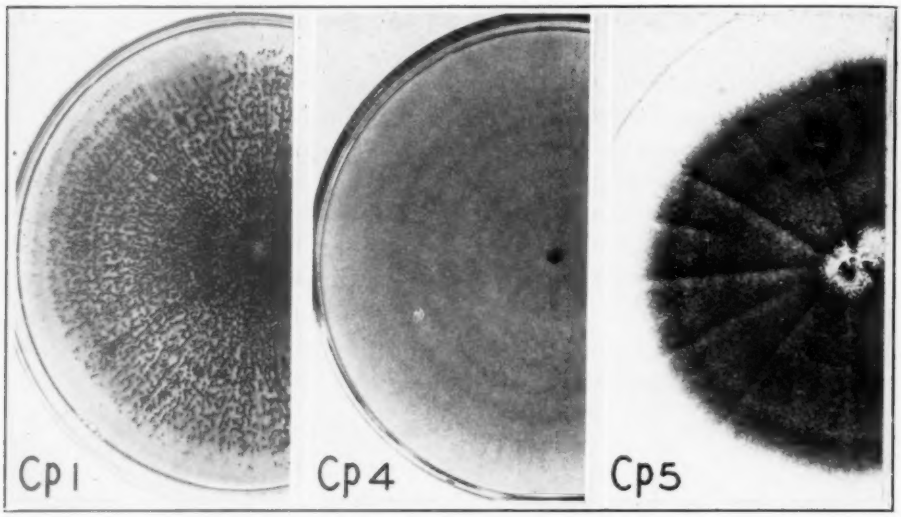

FIG. 1. Appearance of isolates Cp1, Cp4, and Cp5 grown on potato-dextrose agar at 24° C.

single-spore sub-isolates showed a range of 13.8 to 23 × 3 to 4.6 μ, with an average of 18.0 × 3.9 μ. Setae were sparse and stiff; they were always hyaline as compared with the black setae of all other isolates studied. No cultural variation was noted in five successive transfers of 50 single spores for each transfer. Spore production and cultural characters remained constant through successive mass transfers during five years. Albino strains were reported by Caldis and Coons (1) in single-spore cultures of *Physalospora malorum* (Berk.) *Shear* (imperfect stage), *Colletotrichum lindemuthianum* (S. & M.) Bri. & Cav., *Cladosporium fulvum* Cke., and *Septoria apii* (B. & C.) Chester. The variants of *P. malorum* and *S. apii* soon reverted to the parent type; the other two variants retained the albino character for two years. They all sporulated sparsely, they arose as cultural variants and eventually reverted to the parent type; Cp4 was very stable, it was secured as an isolate from naturally infected tomato, and it sporulated abundantly.

Cp5 (Fig. 1) was derived by five successive single-spore transfers from a sector of a strain isolated by W. J. Hooker from naturally infected tomato. Sporulation was practically doubled in amount with each successive transfer. The profusely sporulating strain was, therefore, of the C type as described by Tompkins and Hansen (15). A slight amount of aerial mycelium was formed, and relatively small black sclerotia were produced in such abundance as to aggregate in a crusty layer on the surface of the agar. The spores were uninucleate and were similar in shape and vacuolation to those of Cp4. Measurement of 100 spores from each of five single-spore sub-isolates showed a range of 15 to 25 × 3 to 5 μ with an average of 19.4 × 4.4 μ.

RELATION OF TEMPERATURE TO GROWTH

Isolates Cp1, Cp4, and Cp5 were used. Petri dishes of uniform size containing 15 ml. each of potato-dextrose agar were each inoculated at the center with a 3-mm. disc cut from the periphery of an actively growing

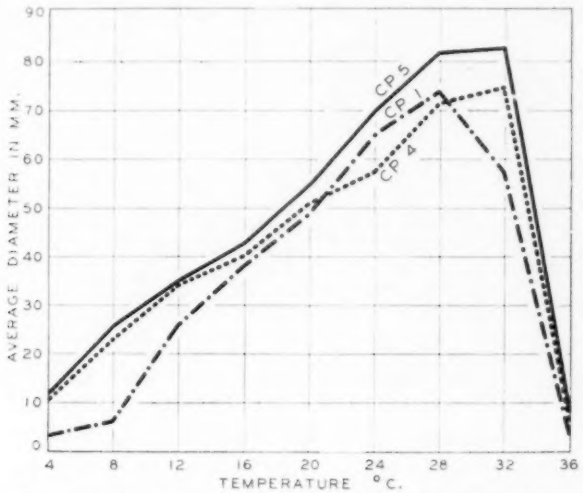

FIG. 2. Relative growth of three isolates after nine days at various temperatures.

culture. Three dishes of each isolate were placed in incubators at 4°, 8°, 12°, 16°, 20°, 24°, 28°, 32°, and 36° C. The plates were wrapped in wax paper to maintain equal moisture conditions. Daily measurements were made of two diameters per colony taken at right angles to each other and averaged. Three experiments gave similar results. The results at nine days from one experiment are reported graphically in figure 2. The MC isolate (Cp1) had a slightly lower optimum than the sporulating isolates. Staling effects were evident with Cp1 at 20° and 24°. When cultures showing no growth at 4° and at 36° were transferred to 24° the normal growth rate for the latter temperature resumed. Sectors varying in size and arrangement of stromata and in sporulation appeared at temperatures from 20° to 32° in Cp1 and Cp5. In all strains stromata appeared when the diameter of the colony reached about 20 mm. Usually spores were formed two to three days after the formation of the stromata.

Relation of Temperature to Spore Germination

Spores from one-week-old cultures of Cp4 and Cp5 were suspended in distilled water and adjusted in concentration to 100 to 150 spores per low power microscopic field (×350). Drops were placed on water agar plates. One hundred spores from each of three plates per temperature were counted. Spores of Cp4 did not germinate at 4° and 36° C. up to eight days; those of Cp5 all germinated at 4° in 8 days and at 36° in two days. Complete or nearly complete germination of spores was reached most promptly with Cp4 and most promptly with both at 20° to 32°. In both isolates spores usually germinated by a single germ tube forming at one end at 4° to 28° and by tubes from both ends at 32° and 36°.

Relation of Carbon Source

A modification of Duggar's solution[3] was used as the basic nutrient in physiologically clean 125-ml. Erlenmeyer flasks to which weighed filter paper cones were added as a substrate for fungus growth. The carbon sources were: glucose, d-levulose, lactose, d-xylose, dextrin, inulin, sucrose, soluble starch, galactose, glycerine, and ethyl alcohol. Twenty-five ml. of a 10 per cent solution of the given carbon source was made up to 50.5 ml. with the basic nutrient. The initial reaction was approximately 4.5; the final reaction was 6 to 7. Each flask was inoculated with 0.15 ml. of a suspension of spores of strain Cp5. In another series 1.7 gm. of agar per 100 ml. was added to the solution and three Petri dishes per treatment poured with 20 ml. of the medium per dish. The plates were inoculated with a 3-mm. loop of spore suspension. All cultures were incubated at 22° C. Three replicates were made for each treatment. Growth in the liquid cultures was determined after 21 days by filtering off the fungus mat and filter paper, drying to a constant weight at 110° C. and subtracting the weight of the filter paper from the total weight. Averages of replicates are presented. Spore production on the agar cultures was estimated by the appearance of spore masses, when visible, or by the amount of spores obtained in drawing a water-filled loop across the surface once. Production of setae was estimated by examination of the culture with a binocular. The results are presented in table 1.

Glucose, dextrin, and soluble starch were good carbon sources in liquid and solid media. Lactose and inulin were better sources on solid than in liquid medium. The poorest sources of carbon were d-xylose and ethyl alcohol. Best sporulation was with inulin, glucose, sucrose, dextrin, and lactose and poorest with d-levulose and soluble starch. No spores occurred in d-xylose, ethyl alcohol, and the control. Formation of setae was usually correlated with spore production, the exception being ethyl alcohol with which there were many setae but no spores. No setae were formed with d-xylose and in the control.

[3] 10.0 ml. 0.5 M KNO_3; 10 ml. 0.25 M KH_2PO_4; 5.0 ml. 0.10 M $MgSO_4$; 0.5 ml. 0.001M $FeCl_3$; 0.005 ml. of a minor element solution consisting of 2.818 gm. H_3BO_3, 0.04 gm. $CuCl_2 . 2H_2O$, 0.03 gm. $ZnCl_2$, and 0.39 gm. $MnCl_2$ per liter; made up to 1000 ml. with distilled water.

TABLE 1.—*Relation of carbon source to growth and sporulation of Colletotrichum phomoides*

Carbon source	Weight of mat in liquid culture	Diameter of colony on agar at 5 days	Relative order as to	
			Sporulation	Formation of setae
	Gm.	*Mm.*		
None	0.00	0.0	None	None
Glucose	0.96	50.0	2	1
D-levulose	0.80	31.0	5	3
Lactose	0.72	40.3	4	2
D-xylose	0.26	0.0	None	None
Dextrin	0.87	50.0	4	4
Inulin	0.67	44.3	1	1
Sucrose	0.82	39.0	3	3
Soluble starch	0.90	32.0	6	5
Galactose	0.00	.....	.....	.....
Glycerine	0.81	.....	.....	.....
Ethyl alcohol	0.13	18.0	None	2

Sporulation on Standard Media

Cp1, Cp4, and Cp5 were compared on media listed in table 2. The best sporulation with all three strains occurred in nutrient dextrose which might well have been due to accessory substances in the peptone and beef extract. Cp1, which sporulated poorly on potato-dextrose agar, produced spores profusely on this medium. By contrast, all 3 strains sporulated poorly in Steinberg's dibasic medium although setae were abundant in Cp4 and Cp5. Elliott's and Richard's media, which differ from Steinberg's in containing monobasic phosphate and a different source of nitrogen, supported good sporulation in Cp4 and Cp5 but not in Cp1. In Leonian's and Czapek's media spore production was moderate in Cp4 and Cp5 and sparse in Cp1.

Relation of Nutrition to Spore Germination

Spores from 14-day cultures of Cp4 and Cp5 were suspended in a 1 per

TABLE 2.—*Comparison of agar media used in study of spore and seta production*

Medium	Grams per 500 ml. of water of				
	Dextrose	Sucrose	$MgSO_4 \cdot 7H_2O$	KH_2HPO_4	Other
Steinberg's Dibasic	.....	25.0	0.3	0.24	1.0 NH_4OH 0.01 $FeCl_3$
Elliott	25.0	.....	0.25	0.68	0.53 Na_2CO_3 0.50 asparagin
Nutrient Dextrose	5.0	.....	.....	.....	1.5 beef extract 5.0 peptone
Leonian	6.13	.....	0.60	0.60	3.13 malt extract
Richards	.....	25.0	1.25	2.5	5.0 KNO_3 0.01 $FeCl_3$
Czapek	.....	.....	0.25	0.5	0.005 $Fe_2(SO_4)_2$ 1.0 $NaNO_3$ 0.25 KCl

cent dextrose solution, 1 per cent peptone solution, saline solution (0.85 per cent sodium chloride), and distilled water at concentrations of about 200 spores per low power field. Drops of a given suspension were placed on clean glass slides within Petri dishes containing 5 ml. of the medium absorbed by filter paper. During incubation at 28° C., 100 spores per drop were counted at frequent intervals. The averages of six replicates are given in table 3. Spores of Cp5 germinated more rapidly than those of Cp4. Most rapid germination occurred in peptone, next in dextrose. Cp5 germinated nearly as rapidly in distilled water as in dextrose, but Cp4 was retarded markedly. Saline solution was decidedly unfavorable.

TABLE 3.—*Influence of nutrient on germination of conidia of Colletotrichum phomoides*

Medium	Strain	Percentage germination at hours indicated								
		3	5	6	8	10	12	22	27	46
Dextrose	Cp4	0	0.0	0.0	0.0	0.0	0.0	4.3	27.1	96.1
	Cp5	0	0.4	0.9	1.0	1.3	1.5	23.3	63.2	90.0
Peptone	Cp4	0	2.0	3.5	6.2	6.5	7.6	28.2	90.0	90.0
	Cp5	0	2.5	4.5	13.5	23.0	37.5	90.0	90.0	90.0
Saline	Cp4	0	0.0	0.4	0.5	0.5	0.5	0.5	0.7	31.0
	Cp5	0	0.0	0.0	0.5	0.8	0.8	1.0	1.5	19.0
Distilled water	Cp4	0	0.0	0.0	0.4	0.4	0.4	0.5	9.1	43.0
	Cp5	0	0.0	0.0	0.8	0.8	1.2	29.0	73.5	90.0

Relation of Reaction of Medium to Growth and Sporulation

Potato-dextrose agar was adjusted to a range of pH values from 2.9 to 9.9. Plates were poured and inoculated with 3-mm. discs from cultures of Cp4 and Cp5 and incubated at 24° C. Growth of both strains was retarded decidedly at pH 2.9, there was moderate growth at 3.9, and good growth at initial pH values from 4.9 to 9.9. No sporulation occurred at 2.9; most abundant sporulation occurred at 3.9; sporulation was slightly less at 4.9 to 6.9; it decreased rapidly above 6.9 and was rare at 9.9.

Relation of Host Substrate to Growth, Sporulation, and Survival

Since concurrent studies showed that *Colletotrichum phomoides* is for the most part a saprophyte and pathogenic chiefly in connection with fruit decay, the relation of host substrate to growth and survival in the soil was studied. In previous sections it was shown, moreover, that the carbon and nitrogen sources and reaction of the substrate had a bearing on the extent of growth and sporulation. Green and ripe fruits, and green and dead stems were cut into small pieces and sterilized in pint bottles alone and in various combinations with soil and sand. They were inoculated with Cp4 and Cp5, incubated at 22° C. for 10 days, and observed. After one month they were set out of doors in November at Madison and left until May. During the winter period temperatures were below freezing for most of the time in December, January, and February.

Soil and sand alone were very poor media for growth. Best growth and sporulation occurred on green and ripe fruit tissue and green stem tissue alone and when mixed with sand. Poorest development occurred on these substrates in soil. Development on dead stems was intermediate whether alone or mixed with sand or with soil.

When the cultures were examined and attempts made to recover the fungus after overwintering, quite the opposite results were secured. The fungus was recovered from each substrate where soil was incorporated, but consistently with sand only when dead stems were used. When the various host tissues were used alone, the fungus was recovered only from dead stems inoculated with Cp5. The slow initial growth of the fungus in substrate mixed with soil appeared to be the best environment wherein the fungus was conditioned most favorably for winter survival.

THE DISEASE CYCLE

Overwintering of the Causal Organism

In the autumn of 1943 naturally infected tomato stems were collected from the field. They bore black, relatively inconspicuous stromata less than 0.5 mm. in diameter scattered irregularly over the surface (Fig. 3). *Colletotrichum phomoides* was isolated in each of 100 fragments tested. A crop of tomatoes was grown on the same field in 1944 and in 1945 with considerable damage from anthracnose. In the spring of 1946 fragments of tomato stems were collected; oftentimes they were partially buried by soil. Of 125 fragments tested, 111 yielded *C. phomoides*. When the stems were washed and placed in a moist chamber, setae and spore masses developed readily on the stromata.

An experiment was performed in this field in 1946 to determine whether the overwintering fungus or inoculum from an outside source was the more important in producing fruit infections later in the season. One thousand tomato plants were set out. Twenty-five plants were inoculated at the blossom stage by atomizing a spore suspension of the non-pigmented strain (Cp4) on the foliage at dusk. Twenty-five plants were inoculated in the same manner at the green-fruit stage. The non-pigmented strain was used to serve as a marker since it had not been obtained in the Madison area and all isolates from overwintered debris were darkly pigmented. Anthracnose developed after late summer rains in the middle of September. About 10 per cent of the fruits were infected throughout the field and the percentage was not higher on the inoculated tomato plants than on the uninoculated ones. Three hundred random isolations, 100 at each collection, were made from infected fruit in inoculated as well as uninoculated rows at two five-day intervals at the peak of the development of the disease. A pigmented isolate was obtained in every case. It was evident, therefore, that inoculum had come from overwintering debris and had not spread from artificially inoculated plants. This experiment was repeated in 1947 with identical results.

In a second experiment in 1947 a row of tomato plants with maturing fruit was inoculated with the non-pigmented strain through incisions in stem and ripe fruit. The plants were then covered with burlap for 40 hours. The non-pigmented strain was not recovered from inoculated stems and only from fruits which had been inoculated. Again all natural infection was from overwintering inoculum.

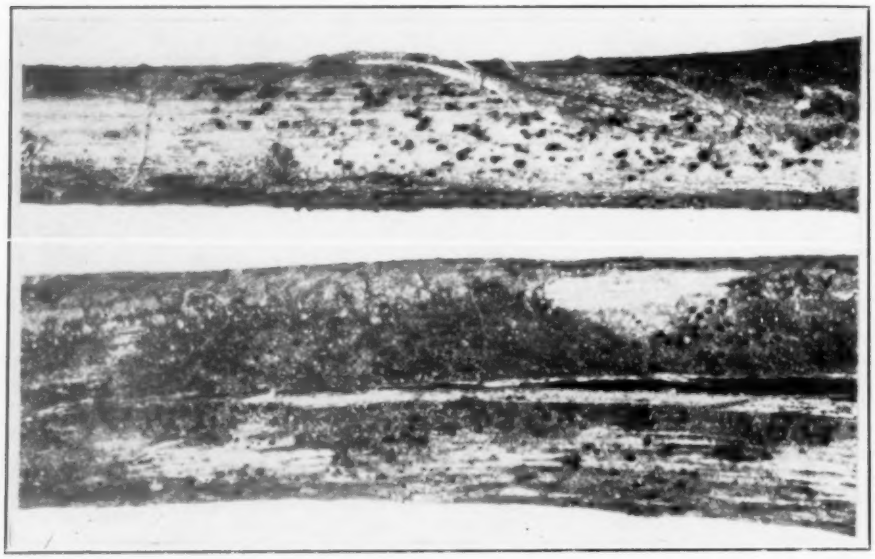

FIG. 3. Stromata of *Colletotrichum phomoides* scattered among fruiting structures of other fungi on dead tomato stems collected in a naturally infested field late in the autumn.

Seed Infection

Sterilized seeds were soaked for 24 hours in spore suspensions of Cp1, Cp4, and Cp5 and sown in sterilized soil. No infection occurred with Cp4 and only rarely did plants show infection with Cp1 and Cp5. Affected plants had small black lesions on the cotyledon, often extending down the cotyledon; or one or both cotyledons showed various degrees of collapse and chlorosis. The presence of the respective strain was confirmed by isolation. From naturally infected fruits 500 seeds were plated without further treatment; seven yielded the anthracnose organism. When 950 seeds from the same fruits were treated with 1 per cent sodium hypochlorite for 10 minutes and plated, none yielded the organism. When 1400 seeds from naturally infected fruits were planted in non-infested soil one plant was found to become infected. It appears, therefore, that the anthracnose organism may be transmitted with seeds from infected fruits.

Foliage Infection

Plants were placed in a moist chamber for two days, inoculated with Cp4 and Cp5, respectively, and kept in the moist chamber for two days

longer. Small brown necrotic spots bordered by chlorotic zones appeared on the cotyledons, which turned yellow, shrivelled, and dropped prematurely. On leaflets, small necrotic spots surrounded by irregular chlorotic areas appeared near the margins. Similar lesions without chlorosis appeared on the stems. In the ordinary greenhouse atmosphere no sporulation occurred on the lesions, but when plants were placed in the moist chamber for four days setae and spores formed. Lesions on a leaflet are shown in figure 4.

FIG. 4. A. Uninoculated tomato leaflet; B. Leaflet from plant inoculated with *Colletotrichum phomoides*. Small necrotic spots from near the margin surrounded by irregular chlorotic areas.

Five-week-old Bonny Best tomato plants were placed in a moist chamber for 24 hours, inoculated, and returned to the moist chamber. Twenty-four plants were removed at 18, 24, 48, 72, and 96 hours, respectively. At each interval six plants were placed in greenhouses at 16°, 20°, 24°, and 28° C., respectively. The duration of the post-inoculation incubation in the moist chamber was important. Only slight development occurred up to 24 hours. The number and size of lesions were greater at 48 and 72 hours and still greater at 96 hours' exposure. The lower leaves and the cotyledons showed

the greatest effect. The enlargement of lesions was checked upon removal of the plants from the moist chamber and the temperature of the greenhouse after removal of plants from the moist chamber had little or no effect upon disease development. Control plants sprayed with distilled water showed no signs of disease. Plants inoculated and placed in the respective greenhouses without moist-chamber treatment remained free from symptoms. In plants inoculated at four to nine weeks after transplanting there was a slightly greater amount of infection on the oldest plants inasmuch as they held more old leaves upon which most of the lesions occurred. Leaves containing lesions were incubated in moist chambers for three days at temperatures from 12° to 32° C. Setae and spores formed most abundantly at 28° and 32°, nearly as abundantly at 24°, and sparsely at 20°, 16°, and 12°. When plants were inoculated and placed in moist chambers at 28° and 16°, respectively, lesions appeared in three days at the high temperature and in 11 days at the low temperature.

Root Infection

White quartz sand was infested with *Colletotrichum phomoides* by incorporation of a pulverized cornmeal-sand mixture which had been sterilized and inoculated with the organism 17 days previously. Tomato seedlings were transplanted to the sand in crocks and watered with Hoagland's nutrient solution. A sand temperature of 28° C. and an air temperature of 24° were maintained for six weeks, when plants were removed and examined. Most plants had small inconspicuous necrotic flecks on the tap root and hypocotyl. Some of the lateral roots were rotted off. In general the injury, even under conditions favorable to the organism, had little retarding effect upon plant growth.

Fruit Infection

Forty green and 40 ripe fruits were inoculated with strains Cp4 and Cp5. Six inoculations were made on each fruit, three by placing a drop of the spore suspension on the uninjured surface, and three by making a puncture wound with a sterile needle through a drop of the spore suspension. The fruits were placed in a moist chamber for 48 hours at about 22° C. The results of the experiment are given in table 4. In no case did infection result from inoculation of the uninjured surface in either green or ripe fruits. However, a high percentage of infection by both strains resulted in both the green and ripe fruits when inoculations were made through wounds. Typical anthracnose lesions were produced by both strains on ripe fruits. Green fruits were slightly less susceptible to infection, and expansion of the lesion, consisting of dark necrotic tissue bordering the wound, was much less than in the ripe fruits.

Penetration of the ripe and green fruits inoculated with strain Cp5 was studied. On uninjured surfaces of both ripe and green fruits, spores germinated well at 24 hours and germ tubes measured 20 to 40 μ in length.

Appressoria formed at the ends of about three-fourths of the germ tubes, but no penetration hyphae were evident. No further development was seen even after 70 hours. On the injured surfaces of the ripe and green fruits, spore germination occurred abundantly after 24 hours, with germ tubes growing towards and into the wound. Appressoria formed on about 50 per cent of the tubes. A few parenchyma cells bordering the wound on green fruits were invaded at this time. At the end of 46 hours the wound had a sunken appearance. The fungus was more advanced in the parenchyma of ripe fruit than green fruit and after 70 hours the lesions on the ripe fruits were 2 to 3 cm. in diameter, compared to 3 or 4 mm. on green fruits. Penetration from cell to cell of the hypodermis of green and ripe fruits was studied in tissues cleared in a solution containing equal parts of acetic acid and ethyl alcohol, and stained with cotton blue in lactophenol.

TABLE 4.—*Infection of wounded and unwounded green and ripe fruits by two strains of Colletotrichum phomoides*

Strain	Ripe Fruits				Green Fruits			
	Wounded		Unwounded		Wounded		Unwounded	
	Inoculated	Infected	Inoculated	Infected	Inoculated	Infected	Inoculated	Infected
	No.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*
Cp4	120	117	120	0	120	97	120	0
Cp5	120	116	120	0	120	101	120	0

The hyphae branched in all directions and penetrated the cell walls readily. The protoplasm of uninvaded parenchyma cells appeared to coagulate in advance of the pathogen.

Related Hosts

Six-week old tomato, eggplant (*Solanum melongena* L. var. *esculentum* Nees.) and pepper (*Capsicum annuum* L.) plants were placed in covered chambers and inoculated by atomizing with a spore suspension of Cp5. Five plants were used for each treatment, one plant serving as a control. The plants were incubated in a moist chamber at approximately 28° C. At six days, tomato plants inoculated with strain Cp5 had many pin-point necrotic spots on the cotyledons, which were chlorotic and curled. Primary leaves were infected predominantly at the margins, showing necrotic tissue surrounded by chlorotic areas. Eggplant cotyledons, which dropped prematurely, were yellowed and had numerous brown necrotic spots. Many dark green necrotic areas occurred on the inoculated leaves, and although small and unexpanded the area of effective photosynthesis was greatly reduced because of their abundance. The leaves yellowed and dropped prematurely. A typical diseased plant is shown in figure 5. The cotyledons of the young pepper plants did not become yellow or drop prematurely, but had numerous pin-point necrotic lesions consisting of collapsed tissue. The leaves had

tiny, brown, necrotic areas at the points where water droplets had persisted. Chlorosis did not occur on the pepper leaf to the extent that it had with tomato and eggplant leaves. Symptoms on pepper are shown in figure 6. Control plants of the three hosts showed no signs of disease. The organism was also isolated from naturally infected pepper fruits grown in a tomato field near Madison in which the disease occurred on tomato.

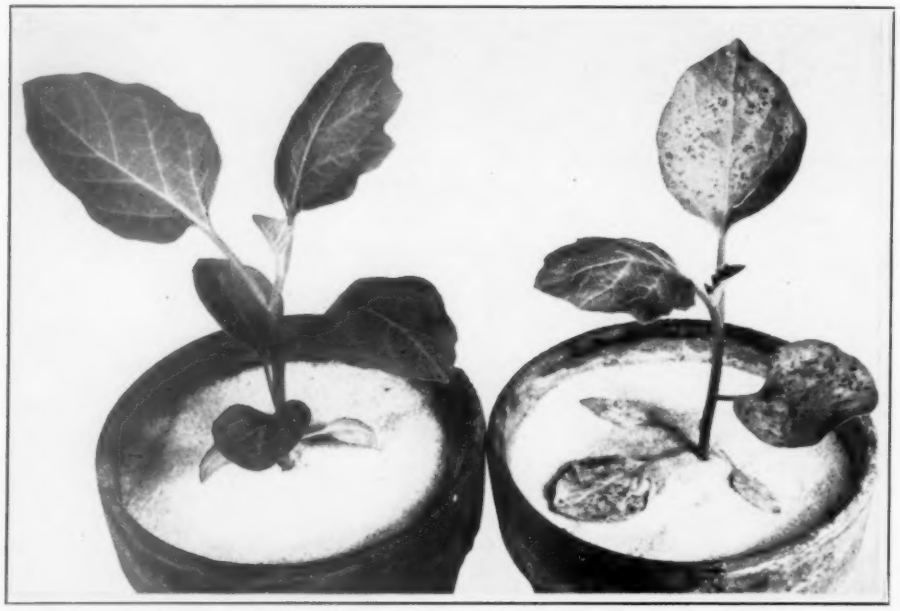

FIG. 5. Left, uninoculated eggplant; right, eggplant inoculated with *Colletotrichum phomoides*. Necrotic spots, chlorosis, and premature dropping occur on cotyledons and leaves.

DISCUSSION AND SUMMARY

The tomato anthracnose organism is important as a pathogen only on ripening tomato fruit, but under favorable conditions it will infect the foliage, stem, and root on which it causes very inconspicuous symptoms and no measurable damage. The fungus grows well as a saprophyte on common media and can use a number of simple and complex carbon compounds as a source of energy. It tolerates a wide range of hydroxyl- and hydrogen-ion concentration in the medium and sporulation occurs over a slightly narrower range. A relatively high temperature (24° to 28° C.) is most favorable for sporulation, germination of spores, growth, and infection. In spite of a rather wide range of adaptability to conditions in pure culture, the fungus dies out rapidly in soil. It is probably not a good soil inhabitant but it survives winter conditions best as stromata on infected plant debris in intimate association with the soil. How long it may remain as a soil invader in association with plant debris has not been determined, but it has been shown to infect fruits of pepper, grape, apple, eggplant, canna, and mango (7, 9),

and has been found on wheat and oats in association with root rot (14). While the fungus may be transmitted with seed and possibly in association with small necrotic lesions on transplants, the chief means of overwintering appears to be as stromata on plant debris. The organism is very mildly pathogenic on plant parts other than the ripening fruits. This is in accord with the report of Younkin and Dimock (17) and contrary to Rolfs (12) who

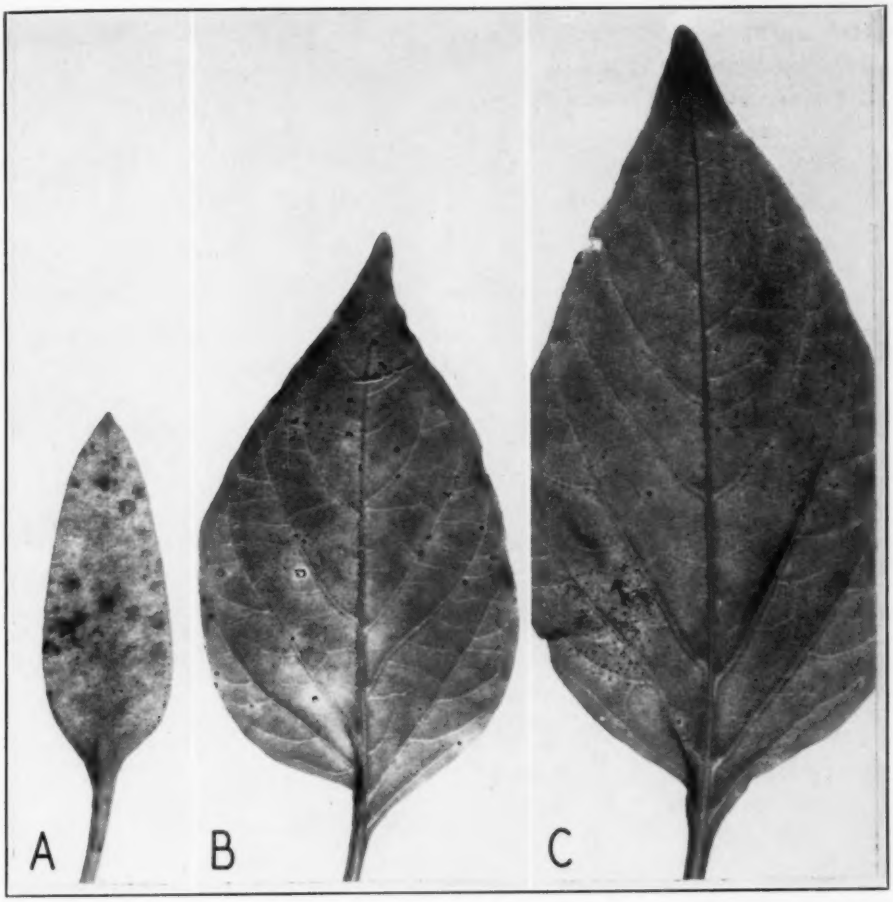

FIG. 6. Symptoms produced on pepper inoculated with *Colletotrichum phomoides*. A, cotyledon; B, young leaf; C, mature leaf. Chlorotic haloes surround lesions on the cotyledon and young leaf, while necrotic flecks occur on the old leaf.

reported earlier that the fungus caused extensive damage to the foliage. Infection of leaves, stems, and roots appears to take place without aid of wounds but injury of fruits is necessary before invasion occurs. It is assumed that wind-blown particles of soil are the chief cause of microscopic wounds on the fruits. When a marker (non-pigmented) strain was inoculated on the foliage and green fruits of plants exposed to natural infection from overwintering debris, all of the fruit infection appeared to come from

the latter source. This indicates that foliage infection is ordinarily a minor source of secondary inoculum. It would also indicate that crop rotation is the primary measure of control to be applied and that the success of chemical prophylaxis will depend upon how well the fungicide adheres to the fruit and protects it from spattering rain bearing soil particles and inoculum.

The mildly pathogenic property of the fungus, except upon maturing storage organs, is parallel with relatively similar situations found in some other anthracnose diseases, particularly those of pea (10), citrus (5), onion (16), and cucurbits (8).

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PHYTOPATHOLOGICAL NOTE

Botryosphaeria ribis, Perfect Stage of the Macrophoma Causing Ripe Rot of Muscadine Grapes.[1]—In 1941 Jenkins[2] reported a ripe rot of muscadine grapes caused by an undetermined species of *Macrophoma.* Further observation of the disease has resulted in the discovery of the perfect stage of this fungus, which is here tentatively referred to *Botryosphaeria ribis* Grossenbacher and Duggar. It might equally as well be placed in *B. berengeriana* DeNot. or other species of *Botryosphaeria. Botryosphaeria* is obviously in need of intensive study and revision. At present it is impos-

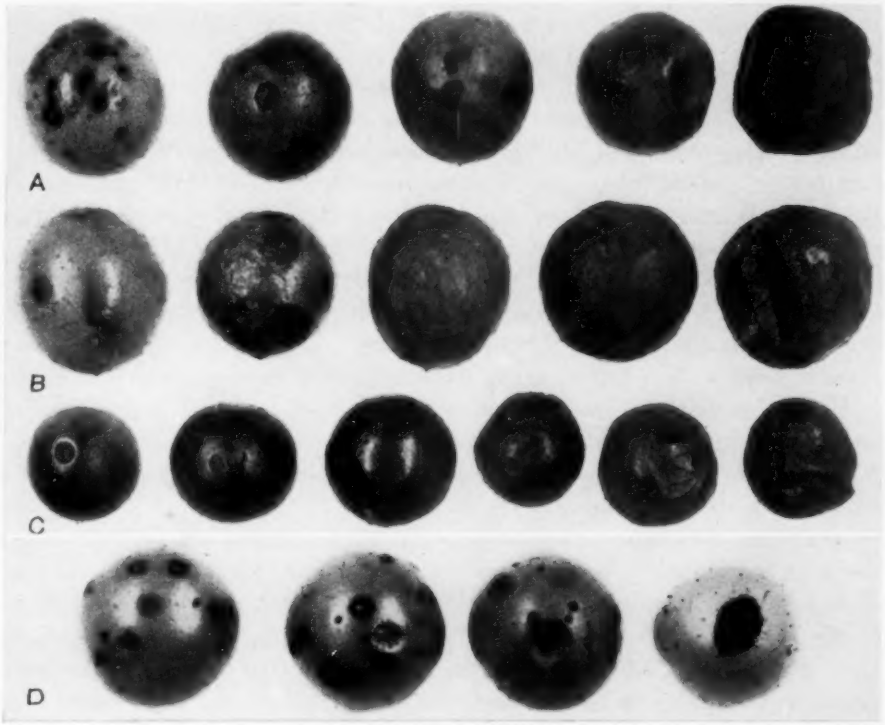

FIG. 1. Ripe rot of muscadine grapes. A. Howard. B. Lucida. C. Thomas. D. Willard. A, B, & C—× 1; D—× 1.2.

sible to determine species limits and the proper application of specific names in the genus.

The ripe rot caused by *Botryosphaeria ribis* appears when the berries reach full size, and it becomes more abundant as they ripen. The first symptoms of the disease are circular, flat or slightly sunken "bird's eye" spots one to four mm. in diameter (Fig. 1). The lesions are dark brown with small, tan or buff-colored centers in which a few *Macrophoma* pycnidia

[1] Paper No. 158, Journal Series, Georgia Experiment Station.

[2] Jenkins, W. A. Diseases of muscadine grapes. In Savage, E. F., *et al.* Further studies with the muscadine grape. Georgia Agr. Exp. Sta. Bul. 217. 1941.

are embedded. Usually this is the most severe expression of the disease. On the most susceptible varieties, however, a brown soft rot may spread over the berry from these lesions. Ultimately the entire berry may be rotted (Fig. 1). In later stages of the rot *Melanconium fuligineum* (Scrib. & Viala) Cav. often enters the berries and may be responsible for some of the damage. Berries in which the rot spreads drop from the vines. They shrivel slightly but do not form mummies. They are finally reduced to dry, hollow shells with pycnidia abundant over the entire surface.

Upon the basis of present evidence this disease appears to be of minor importance. Many muscadine varieties are almost immune. The disease is most severe on Thomas and, to a lesser degree, on Lucida, Howard, and Dulcet. Hunt, the chief commercial variety in Georgia, along with Creek, Scuppernong, Irene, and Yuga, is highly resistant. For example, in 1946 counts of the berries that dropped from two vines of each variety from the first of August to harvest time showed that on Hunt the disease was present on only 0.39 per cent of the fallen berries and seemed responsible for only 0.06 per cent of the drop. On Thomas it was present on 25.45 per cent of the fallen berries and seemed responsible for 11.31 per cent of the drop. Only 12.38 per cent of the total crop dropped from these vines, however, and it was estimated that even on Thomas only a 1.40 per cent crop loss could be attributed to berry drop resulting from infection with *Botryosphaeria ribis*. Control measures are considered unnecessary. *B. ribis*, along with *Melanconium fuligineum*, *Alternaria* sp., *Glomerella* sp., *Diaporthe* sp., and *Pestalozzia* sp. has been isolated also from blighted peduncles of flower clusters and berry clusters. On the peduncles it may be a secondary organism. Nevertheless, this aspect of the disease must be investigated further before a final estimate of its importance can be made.

Only the *Macrophoma* pycnidial stage of the fungus has been found on diseased berries. The pycnidia were spherical and measured 153–197 μ in diameter. The pycnospores were hyaline, unicellular, narrowly elliptical to ovoid, and rounded at either end. They measured $14.0–25.2 \times 5.6–8.4\ \mu$ (Av. $25 = 19.6 \times 6.6\ \mu$). Pycnidia filled with viable pycnospores have been found in the spring on berries that had dropped from the vines during the preceding season. The fungus, therefore, is capable of surviving the winter in the pycnidial stage. Pycnospores from an over-wintered berry measured $16.8–24.6 \times 5.6–7.8\ \mu$ (Av. $25 = 20.9 \times 6.4\ \mu$). Isolates of the fungus from rotted berries rarely fruited on agar media. Most of them, however, were induced to fruit on sterile grape stems partially immersed in water in culture tubes. Pycnospores formed under these conditions were elliptical to fusiform. Variations among measurements of pycnospores produced in culture by several isolates were as follows: $16.8–21.0 \times 5.0–7.0\ \mu$ (Av. $25 = 19.0 \times 5.8\ \mu$); $18.2–29.4 \times 5.0–5.9\ \mu$ (Av. $25 = 22.1 \times 5.5\ \mu$); $19.6–28.0 \times 3.6–5.6\ \mu$ (Av. $25 = 22.8 \times 4.9\ \mu$); $19.6–28.0 \times 5.3–9.8\ \mu$ (Av. $25 = 23.0 \times 7.0\ \mu$).

The perfect stage was obtained by inoculating sterile grape stems in culture tubes with isolates of the fungus and overwintering the inoculated

stems out-of-doors. Pycnidia, spermogonia, and ascocarp initials were produced in botryose stromata on these stems during late fall and winter. All three stages have been found associated in a single stroma. Although the pycnidia on berries were separate, those on the stems were usually grouped in stromata and would be referred to *Dothiorella*. Mature ascocarps were produced in the stromata the following spring. They were spherical and measured 172–315 μ in diameter. The locule was filled with a mass of pseudoparaphyses and asci. The asci were cylindrical, 8-spored, possessed a thick, two-layered wall, and measured 102.4–156.8 $\times$ 17.6–24.0 μ. The ascospores were hyaline, unicellular, ovoid to elliptical, and measured 19.6–30.8 $\times$ 8.4–11.2 μ (Av. 25 = 24.9 $\times$ 10.3 μ). Isolates derived from ascospores were identical with those obtained from rotted berries bearing the *Macrophoma* pycnidia.

The relationship of the *Botryosphaeria* perfect stage on over-wintered stems with the *Macrophoma* imperfect stage associated with berry rot is based on the morphologic connection of the pycnidia and ascocarps on grape stems and upon the fact that isolates from both sources are similar in culture. Inoculations have been inconclusive because of natural infection of berries in the field with both *Botryosphaeria ribis* and *Melanconium fuligineum*.—E. S. LUTTRELL, Georgia Experiment Station, Experiment, Georgia.

BOOK REVIEW

WOLF, F. A. and F. T. WOLF. *The Fungi.* Volume I. i–viii and 438 pp., 153 figs.; Volume II. i–xxii and 538 pp., 82 figs. John Wiley and Sons, New York; Chapman and Hall, Ltd., London. 1947. Vol. I, $6.00; Vol. II, $6.50.

This treatise on fungi will serve, as the authors intended, for both a text book and a reference. It will probably fill most of the requirements of the undergraduate student and the teacher. It will also be valuable to the research worker, who, through excessive specialization, may have been unable to keep up with recent mycological advances. The subject matter is attractively presented, is easy to read, and is abundantly illustrated. The occasional quip or jest appearing in the text adds zest to the reading. Each chapter contains a résumé of the matters just discussed, or a summary of relevant implications.

Volume I opens with a short chapter concerned with the history of mycology. This is followed by an outline of modern isolation and culture methods. Of interest to most readers and of great value to the student is a chapter dealing with the principles of fungal taxonomy and classification. The remainder of the volume consists of chapters individually dealing with Myxomycetes, Phycomycetes, Ascomycetes, Basidiomycetes, and Fungi Imperfecti. For each class of fungi, the authors provide a description of its distinguishing features supplemented by descriptions of the principal orders and families. To some the lack of generic keys may seem a fault, but, since attention is drawn to detailed monographic studies dealing with individual groups of fungi, this fault is not serious. In any case, the inclusion of detailed keys would unnecessarily add to the volume and cost of the treatise.

Volume II, devoted to an account of the various activities of fungi, appears to be more suitable for the advanced student of fungi than for the undergraduate. The subject matter consists of chapters dealing with fungal nutrition, enzymatic action, respiration, biochemistry, and the effects of temperature, radiation, reaction of substrate, and association. Detailed accounts are given of spore dissemination and germination, host penetration, and physiologic specialization and variation. In other chapters, the authors discuss such subjects as mycorrhizae, genetics of fungi, poisonous and edible fungi, medical mycology, geographic distribution of fungi, mycology in relation to plant pathology, soil fungi, fungus-insect relationships, marine fungi, and fossil fungi. Many of these subjects are seldom dealt with in other texts and their inclusion adds interest and value to the volume. Not to be overlooked is the excellent author and subject index in both volumes.

It is apparently extremely difficult, in a monumental work of this kind, to avoid the intrusion of at least some errors and only the principal ones are referred to here. For instance, the alternative use of "swarmers," "swarm spores" and "zoospores" without an indication of synonymy may lead to some confusion of the uninitiated reader. Similarly, the persistent misspelling of the names Christensen and Rodenhiser in both text and citations may put difficulties in the way of a student who attempts to locate these authors in library indices. The occasional misspelling of other surnames is obviously a typographical error. The illustrations, though good, are occasionally placed too far away (once 6 pages and once 10 pages away) from the subject discussed in the text. In the discourse on the genetics of fungi, an illustration said to show sex segregation in the second nuclear division actually illustrates segregation during the third division. *Morchella esculenta,* initially referred to as a desirable edible fungus, is included, figured, and described among notoriously poisonous fungi. A considerable number of lesser errors appear, but to the reader they are not misleading and will no doubt be corrected in further editions.

It is problematical whether the average student can afford the price charged for each volume. In the light of the intrinsic value of the treatise and the present cost of printing, the price may not be excessive. The cost may be, however, prohibitive to the student, especially when his other necessary book expenses are taken into account.—JOHN E. MACHACEK, Dominion Rust Research Laboratory, Winnipeg, Manitoba, Canada.

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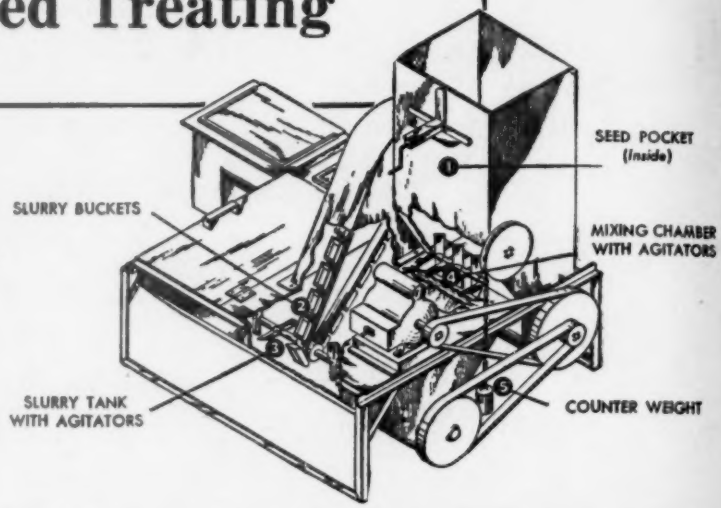

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